SURROGATE MARKERS OF NEUROPATHIC PAIN

Field of the Invention

[0001] The invention is in the fields of neurology and pharmacology. The invention generally relates to methods of evaluating neuropathic pain and to methods of evaluating biological activity of drugs or drug candidates for treating neuropathies.

Background of the Invention

[0002] Painful neuropathies are characterized by spontaneous and/or abnormal stimulus-evoked pain such as allodynia or hyperalgesia. Symptoms of neuropathic pain often include spontaneous cramping, burning, or shooting pain, or pain caused by normally innocuous stimuli. Neuropathic pain has a neurogenic origin, i.e., it is initiated or caused by a primary lesion or dysfunction in the peripheral or central nervous system (see, e.g., Merskey and Bogdik (1994) Classification of Chronic Pain: Descriptions of Chronic Pain Syndromes and Definitions of Pain Terms, 2nd ed., Seattle: IASP Press). Neuropathic pain can occur as a result of nerve damage due to infectious agents (e.g., herpesviruses), metabolic diseases (e.g., diabetes), neurodegenerative diseases (e.g., multiple sclerosis), nerve injury (e.g., amputation or cancer-induced nerve compression), etc. Current pharmacologic and nonpharmacologic therapies for chronic neuropathic pain provide only partial relief and the outcomes vary widely in individual patients.

[0003] Conditions affecting the peripheral nervous system create pathophysiologic changes such as loss of small sensory fibers and/or demyelination. Such changes can be histologically observed in the skin. Indeed, histological

evaluation of skin biopsies has become an accepted method for assessing peripheral nerve status in patients with neuropathic pain or peripheral neuropathy (Griffin et al. (2001) Curr. Opin. Neurol., 14:655-659). This approach allows one to evaluate the progression of nerve damage in disease and regeneration/re-innervation with treatment. Counting criteria include epidermal nerve fiber density, the number of fibers crossing the dermal-epidermal junction, etc. Skin biopsies can be performed in multiple sites over time, so that a spatiotemporal profile of epidermal innervation can be assessed. However, histological analysis of skin biopsies is a laborious and time-consuming procedure.

[0004] Therefore, there exists a need in the art to develop new methods for treatment and assessment of neuropathic pain and peripheral neuropathy.

SUMMARY OF THE INVENTION

[0005] The present invention results from the realization that skin biopsy samples can be nonhistologically evaluated for expression of gene(s) that reflect the neuropathic pain status ("surrogate markers of neuropathic pain"). The expression of such genes can be measured in skin biopsy homogenates in a rapid and quantitative manner. If the expression of the gene(s) in skin punch biopsy samples correlates with the beneficial effect of the drug or drug candidate on neuropathic pain or peripheral neuropathy, then the read-out represents a surrogate marker of drug activity associated with the reduction in neuropathic pain and/or peripheral neuropathy ("surrogate marker of neurotrophic activity"). Furthermore, gene expression in skin punch biopsy samples can be used as a read-out of in vivo biological activity of a drug or drug candidate regardless of the neuropathic pain status ("biomarker of in vivo

biological activity of a neurotrophic agent" or "biomarker of a neurotrophic agent" for short).

[0006] In one aspect, the invention provides methods of identifying surrogate markers of neuropathic pain. The methods of identifying a surrogate marker of neuropathic pain include:

(a) obtaining a first skin biopsy sample under conditions of neuropathic pain;

(b) obtaining a second skin biopsy sample under conditions of substantially no neuropathic pain;

(c) preparing tissue extracts from the first and the second samples; and

(d) determining an amount of at least one nucleic acid or protein in the tissue extracts.

A difference between the amount of the nucleic acid or the protein in the first sample and the amount of the same nucleic acid or protein in the second sample indicates that the nucleic acid or the protein is a surrogate marker of neuropathic pain.

[0007] In another aspect, the invention provides methods of evaluating the level of neuropathic pain using such surrogate markers. The methods of evaluating the level of neuropathic pain using surrogate markers of neuropathic pain include:

(a) obtaining a first skin biopsy sample under conditions of neuropathic pain;

(b) obtaining a second skin biopsy sample under conditions of substantially no neuropathic pain;

(c) preparing tissue extracts from the first and the second samples; and

(d) determining an amount of at least one nucleic acid or protein in the tissues, the nucleic acid or the protein being a surrogate marker of neuropathic

pain.

A difference between the amount of the nucleic acid or the protein in the first sample and the amount of the same nucleic acid or protein in the second sample indicates the level of neuropathic pain.

[0008] In another aspect, the invention provides methods of evaluating neurotrophic activity of a compound or composition, for example, in evaluating the effect of a compound of composition on the level of neuropathic pain. The methods include:

(a) administering the compound or composition to the mammal having neuropathic pain;

(b) obtaining at least one skin biopsy sample from the mammal;

(c) preparing a tissue extract from the skin biopsy sample; and

(d) determining an amount of at least of one surrogate marker of neuropathic pain that is nucleic acid or protein in the tissue extract.

A difference in the amount of the nucleic acid or protein determined in step (d) and the amount of the same nucleic acid or protein expressed in the absence of the compound or composition indicates the level of efficacy of the compound or composition on neuropathic pain.

[0009] In another aspect, the invention provides methods of identifying biomarkers of in vivo biological activity of a neurotrophic agent and methods of evaluating in vivo biological activity of a neurotrophic agent using such biomarkers. The methods of identifying biomarkers of in vivo biological activity of a neurotrophic agent include:

(a) administering the agent to a mammal;

(b) obtaining at least one skin biopsy sample from the mammal;

(c) preparing a tissue extract from the skin biopsy sample; and

(d) determining an amount of at least one nucleic acid or protein in the tissue extract.

A difference in the amount of the nucleic acid or protein determined in step (d) and the amount of the same nucleic acid or protein expressed in the absence of the agent indicates that the nucleic acid or the protein is a biomarker of in vivo biological activity of the agent.

[0010] In another aspect, the invention provides methods of evaluating in vivo biological activity of a neurotrophic agent using biomarkers of in vivo biological activity of such an agent. The methods of evaluating in vivo biological activity of a neurotrophic agent include:

(a) administering the agent to a mammal;

(b) obtaining at least one skin biopsy sample from the mammal;

(c) preparing a tissue extract from the skin biopsy sample; and

(d) determining an amount of at least one nucleic acid or protein in the tissue extract.

A difference in the amount of the nucleic acid or protein determined in step (d) and the amount of the same nucleic acid or protein expressed in the absence of the agent indicates that the agent is biologically active.

[0011] In illustrative embodiments, the neurotrophic agent being evaluated is artemin (also known as neublastin or enovin), a member of the glial-cell-line-derived

neurotrophic factor (GDNF) family.

[0012] Exemplary nucleotide and/or amino acid sequences of human and rat surrogate markers of neuropathic pain, surrogated markers of neurotrophic activity and biomarkers of in vivo biological activity of neurotrophic agents are also provided (see Table 1).

Table 1.

Group No.	SEQ ID NOs:	Preferred SEQ ID NOs:	Category*	Type	Species	Table No.
I	1-308	1-42	SMP	DNA	Rat	Table 2
II	309-470	309-333	SMP	Protein	Rat	Table 3
III	471-630	471-493	SMP	DNA	Human	Table 4
IV	631-790	631-653	SMP	Protein	Human	Table 5
V	791-897	791-814	SMN	DNA	Rat	Table 6
VI	898-962	898-914	SMN	Protein	Rat	Table 7
VII	963-1038	963-979	SMN	DNA	Human	Table 8
VIII	1039-1114	1039-1055	SMN	Protein	Human	Table 9
IX	1115-1163	1115-1120	BMN	DNA	Rat	Table 10
X	1164-1178	1164-1166	BMN	Protein	Rat	Table 11
XI	1179-1207	1179-1182	BMN	DNA	Human	Table 12
XII	1208-1236	1208-1211	BMN	Protein	Human	Table 13

* SMP - surrogate marker of neuropathic pain;
SMN - surrogate marker of neurotrophic activity;
BMN - biomarker of a neurotrophic agent.

[0013] Various embodiments of the invention are set forth in the following description or will be understood from the description.

BRIEF DESCRIPTION OF THE FIGURES

[0014] Figure 1 shows results of a TaqMan™ analysis of gene expression of rc_AA818804_at (SEQ ID NO:18 and SEQ ID NO:799) in the L4 dermatome of rats subjected to spinal nerve ligation injury (SNL) and treament with artemin. The gene is expressed at a low level before injury, at a higher level following injury, and at a near-normal level after injury and treatment with artemin.

[0015] Figure 2 shows results of a TaqMan™ analysis of gene expression of X14812_at (SEQ ID NO:37 and SEQ ID NO:813) in the L4 dermatome of rats subjected to SNL and treament with artemin. The gene is expressed at a low level before injury, at a higher level following injury, and at a near-normal level after injury and treatment with artemin.

[0016] Figure 3 shows results of a TaqMan™ analysis of gene expression of rc_AA818120_at (SEQ ID NO:31 and SEQ ID NO:808) in the L4 dermatome of rats subjected to SNL and treament with artemin. The gene is expressed at a low level before injury, at a higher level following injury, and at a near-normal level after injury and treatment with artemin.

[0017] Figure 4 shows results of a TaqMan™ analysis of gene expression of rc_AA946094_at (SEQ ID NO:2 and SEQ ID NO:791) in the L4 dermatome of rats subjected to SNL and treament with artemin. The gene is expressed at a low level before injury, at a higher level following injury, and at a near-normal level after injury and treatment with artemin.

[0018] Figure 5 shows results of a TaqMan™ analysis of gene expression of

X07314cds_at (SEQ ID NO:11 and SEQ ID NO:796) in the L4 dermatome of rats subjected to SNL and treament with artemin. The gene is expressed at a low level before injury, at a higher level following injury, and at a near-normal level after injury and treatment with artemin.

[0019] Figure 6 shows results of a TaqMan™ analysis of expression of gene M27151_at (SEQ ID NO:22 and SEQ ID NO:801) in the L4 dermatome of rats subjected to SNL and treament with artemin. The gene is expressed at a low level before injury, at a higher level following injury, and at a near-normal level after injury and treatment with artemin.

[0020] Figure 7 shows results of an Affymatrix analysis of expression of gene rc_AI072712_at (SEQ ID NO:1118) in the L4 dermatome of rats subjected to SNL and treament with artemin. Regardsless of injury state, this gene is expressed at a relatively high level in the vehicle-treated samples, and at a much reduced level following treatment with artemin.

DETAILED DESCRIPTION OF THE INVENTION

[0021] In the experiments leading to the present invention, rats were subjected to unilateral spinal nerve ligation (SNL) to induce unilateral neuropathic pain. Following SNL, some rats were systemically administered artemin, a neurotrophic factor shown to reduce neuropathic pain (Gardell et al. (2003) Nature Med., 9(11):1383-1389). The induced neuropathic pain was assessed using behavioral tests. Skin samples were then obtained bilaterally and tissue extracts were prepared. RNA from these tissue extracts was subjected to Affymetrix GeneChip™ expression analysis to determine gene expression profiles in various samples.

[0022] The heterogeneity of tissues usually makes it difficult to detect small changes in transcription in tissue samples, especially if the changes are restricted to small subpopulations of cells or are a result of indirect effects. Despite this difficulty, the present invention is based, in part, on the discovery and demonstration that detectable changes in gene expression in skin biopsy homogenates reflect the neuropathic pain status.

[0023] In particular, the methods of the invention may be used to identify genes whose expression levels correlate with neuropathic pain (surrogate markers of neuropathic pain). The invention may be also used to identify a subset of these genes whose expression levels are at least partially normalized by the artemin treatment (surrogate markers of neurotrophic activity). The invention may be used to identify an additional set of genes whose expression levels correlate with the presence of biologically active artemin regardless of the neuropathic pain status (biomarkers of a neurotrophic agent).

Surrogate markers of neuropathic pain

[0024] The invention provides a method of identifying a surrogate marker of neuropathic pain in a mammal, comprising:

(a) obtaining a first skin biopsy sample under conditions of neuropathic pain;

(b) obtaining a second skin biopsy sample under conditions of substantially no neuropathic pain;

(c) preparing tissue extracts from the first and the second samples; and

(d) determining an amount of at least one nucleic acid or protein in the tissue extracts;

wherein a difference between the amount of the nucleic acid or the protein in the first sample and the amount of the same nucleic acid or protein in the second sample indicates that the nucleic acid or the protein is a surrogate marker of neuropathic pain. In some embodiments, the amount of the nucleic acid or the protein in the first sample will differ from the amount of the same nucleic acid or protein in the second sample by, for example, 2, 3, 4, 5, 8, 10, 20, 30, 40, 50, 80, 100-fold, or more. The difference (also referred to as "fold-change") indicates a correlation of the downregulation or upregulation of the relevant gene and neuropathic pain. The greater the fold-change in expression and/or the higher the degree of correlation with neuropathic pain, the more preferable the nucleic acid or protein is as a surrogate marker of neuropathic pain.

[0025] The first and the second samples can be obtained from the same mammal or from different mammals. For example, the first and second samples can be obtained from the same mammal from different regions of the skin, one region affected by neuropathic pain or peripheral neuropathy, and the other region not affected by pain or neuropathy. In another example, the first and second samples can be obtained from the same region of the skin in the same mammal but at different times. For example, a first sample can be collected prior to inducing neuropathic pain and the second sample is obtained following induction of neuropathic pain. In yet another example, the first sample can be collected from the region affected by neuropathic pain, and the second sample is obtained from the same region following treatment. Alternatively, the first and second samples can be obtained from different mammals and the amounts of a nucleic acid or protein are compared with reference to

a common control using statistical analysis.

[0026] Illustrative methods of identifying a surrogate marker of neuropathic pain in rats are provided in the Examples. 308 rat nucleic acids (Table 2) were identified following these illustrative methods. Corresponding protein sequences and human orthologues were then identified using publicly available databases such as GenBank™. 162 rat protein sequences (Table 3), 160 human nucleic acid sequences (Table 4), and 160 human protein sequences (Table 5) were identified in this manner.

[0027] The invention provides a method of evaluating the level of neuropathic pain in a mammal, comprising:

(a) obtaining a first skin biopsy sample under conditions of neuropathic pain;

(b) obtaining a second skin biopsy sample under conditions of substantially no neuropathic pain;

(c) preparing tissue extracts from the first and the second samples; and

(d) determining an amount of at least one nucleic acid or protein in the tissues, the nucleic acid or the protein being a surrogate marker of neuropathic pain;

wherein a difference between the amount of the nucleic acid or the protein in the first sample and the amount of the same nucleic acid or protein in the second sample indicates the level of neuropathic pain. In some embodiments, the amount of the nucleic acid or the protein in the first sample will differ from the amount of the same nucleic acid or protein in the second sample by, for example, 2, 3, 4, 5, 8, 10, 20, 30, 40, 50, 80, 100-fold, or more. The difference ("fold-change") in the expression levels of a relevant surrogate marker of neuropathic pain correlates with the level, or degree,

or neuropathic pain. Generally, surrogate markers of neuropathic pain that exhibit greater fold-change values indicate a higher degree of neuropathic pain.

[0028] The first and the second samples can be obtained from the same mammal or from different mammals as described herein.

[0029] In some embodiments, the surrogate marker of neuropathic pain is a nucleic acid. In illustrative embodiments, the nucleic acid comprises a nonredundant subsequence of any one of the rat nucleotide sequences of SEQ ID NOs:1-308, preferably SEQ ID NOs:1-42. In other illustrative embodiments, a surrogate marker of neuropathic pain is a nucleic acid that comprises a nonredundant subsequence of any one of the human nucleotide sequences of SEQ ID NOs:471-630, preferably SEQ ID NOs:471-493.

[0030] In some embodiments, the surrogate marker of neuropathic pain is a protein. In illustrative embodiments, the protein comprises a nonredundant subsequence of any one of the rat protein sequences of SEQ ID NOs:309-470, preferably SEQ ID NOs:309-333. In other illustrative embodiments, a surrogate marker of neuropathic pain is a protein that comprises a nonredundant subsequence of any one of the human protein sequences of SEQ ID NOs:631-790, preferably SEQ ID NOs:631-653.

[0031] Conditions in which neuropathic pain may occur, and therefore may require assessment in the course of diagnosis or treatment, include but are not limited to: traumatic (including iatrogenic) nerve injury, ischemic neuropathy, nerve compression/entrapment, polyneuropathy (hereditary, metabolic, toxic, inflammatory; infectious, paraneoplastic, nutritional, in amyloidosis and vasculitis), plexus injury root

compression, stump and phantom pain after amputation, herpes zoster/postherpetic neuralgia, trigeminal and glossopharyngeal neuralgia, cancer-related neuropathy (due to neural invasion of the tumor, surgical nerve damage, radiation-induced nerve damage, chemotherapy-induced neuropathy), stroke (infarct or hemorrhage), multiple sclerosis, spinal cord injury, syringomyelia/syringobulbia, epilepsy, and space-occupying lesions. Examples of specific disorders include diabetic neuropathy, sensory neuropathy of AIDS and antiretroviral toxic neuropathy, idiopathic small fiber neuropathy, leprosy, Fabry disease. Additionally, the method of assessing neuropathic pain may be used to assess induced neuropathic pain in experimental animals, e.g., SNL-induced neuropathic pain in rats as described in the Examples.

[0032] Assessment of pain with the methods of the invention may be conducted in the course of pharmacological and/or nonpharmacological treatments. Nonpharmacological treatments of neuropathic pain include transcutaneous electrical nerve stimulation, spinal cord stimulation, motor cortex stimulation, deep brain stimulation, decompression, neuroma removal, neurotomy, glycerol injection, radiofrequency nerve/root lesion, dorsal root entry zone lesion, and cordotomy.

Surrogate markers of neurotrophic activity

[0033] A subset of surrogate markers of neuropathic pain is expected to be normalized as a result of a treatment with a compound or a composition that reduces neuropathic pain.

[0034] Accordingly, the invention provides a method of evaluating the effect of a compound or composition on the level of neuropathic pain in a mammal, comprising:

(a) administering the compound or composition to the mammal having

neuropathic pain;

(b) obtaining at least one skin biopsy sample from the mammal;

(c) preparing a tissue extract from the skin biopsy sample; and

(d) determining an amount of at least one nucleic acid or protein in the tissue extract, the nucleic acid or the protein being a surrogate marker of neuropathic pain;

wherein a difference in the amount of the nucleic acid or protein determined in step (d) and the amount of the same nucleic acid or protein expressed in the absence of the compound or composition indicates the level of efficacy of the compound or composition on neuropathic pain.

[0035] The amount of a nucleic acid or protein expressed in the absence of the compound or composition can be determined by any suitable method. In one method, the amount of the nucleic acid or protein in the test sample is compared to the amount of the same nucleic acid or protein in another sample obtained in the absence of the compound or composition from the same mammal or from different mammals. The control sample may be collected before, during, or after the analysis. In another method, the amount of the nucleic acid or protein in the test sample is compared to that of one or more internal references. An internal reference is a nucleic acid or a protein whose expression levels under given conditions are known. Most typically, the reference is a gene that remains relatively constant under various conditions such as a housekeeping gene, e.g., actin or GAPDH.

[0036] In some embodiments, the amount determined in step (d) will differ from the amount of the same nucleic acid or protein expressed in the absence of the

compound or composition by, for example, 2, 3, 4, 5, 8, 10, 20, 30, 40, 50, 80, 100-fold, or more. The "normalization" of the expression level of a relevant surrogate marker of neuropathic pain towards the baseline expression level as in normal conditions (substantially no neuropathic pain) indicates that the compound or composition reduces neuropathic pain. The difference in expression levels under conditions of neuropathic pain and upon "normalization" ("fold-change-back") indicates the level of neurotrophic activity of the compound or composition being evaluated. Generally, the greater fold-change-back values indicate that the compound or composition is expected to exhibit greater efficacy in treating neuropathic pain. Although greater fold-change-back values are preferred, it is also preferred that a fold-change-back value for a particular surrogate marker of neuropathic pain does not substantially exceed a corresponding fold-change value for the marker.

[0037] Illustrative methods of evaluating the effect of a compound or composition on the level of neuropathic pain in rats are provided in the Examples. 107 rat nucleic acids (Table 6) were identified following these methods. Corresponding protein sequences and human orthologues were then identified using publicly available databases such as GenBank™. 65 rat protein sequences (Table 7), 76 human nucleic acid sequences (Table 8); and 76 human protein sequences (Table 9) were identified in this manner.

[0038] In some embodiments, the surrogate marker of neurotrophic activity is a nucleic acid. In illustrative embodiments, the nucleic acid comprises a nonredundant subsequence of any one of the rat nucleotide sequences of SEQ ID NOs:791-897, preferably SEQ ID NOs:791-814. In other illustrative embodiments, a

surrogate marker of neurotrophic activity is a nucleic acid that comprises a nonredundant subsequence of any one of the human nucleotide sequences of SEQ ID NOs:963-1038, preferably SEQ ID NOs:963-979.

[0039] In some embodiments, the surrogate marker of neurotrophic activity is a protein. In illustrative embodiments, the protein comprises a nonredundant subsequence of any one of the rat protein sequences of SEQ ID NOs:898-962, preferably SEQ ID NOs:898-914. In other illustrative embodiments, the surrogate marker of neurotrophic activity is a protein that comprises a nonredundant subsequence of any one of the human protein sequences of SEQ ID NOs:1039-1114, preferably SEQ ID NOs:1039-1055.

[0040] In some embodiments, the compound or composition to be evaluated is or comprises a neurotrophic agent. "Neurotrophic agent" is a compound that has neurotrophic activity, i.e., it affects generation, survival, growth, or maintenance of normal physiological function of neurons. Neurotrophic activity can be evaluated/measured by one or more methods known in the art, for example:

(1) RET kinase receptor activation ELISA (KIRA) (Milbrandt et al. (1998) Neuron, 20:245; Sadick et al., 1996, Anal. Biochem., 1996. 235(2):207);

(2) choline acyteltransferease enzymatic assays (Leibrock et al.(1989) Nature, 341:149;

(3) ^{3}H-dopamine uptake assay with dopaminergic neurons (Lev-Fen et al. (1993) Science, 260:1130; or

(4) rat pheochromocytome cell line PC12 assays (Ernfors et al. (1991) Nature, 350:1756; Darling et al. (1984) Methods for preparation and assay of nerve

growth factor", Cell Culture Methods for Molecular and Cellular Biology, vol. 4 (eds. Barnes et al.), pp.79-83, Alan R. Liss, New York; Bradshaw (1978) Ann Rev. Biochem, 47:191).

[0041] In illustrative embodiments, the neurotrophic agent being evaluated is artemin. Other examples of neurotrophic agents include neurotrophic factors such as other members of the GDNF family (e.g., GDNF, neurturin, persephin), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neutrotrophin-3 (NT-3), leukocyte migration inhibitory factor (LIF), interleukin 6 (IL6), basic fibroblast growth factor (bFGF), midkine, neutrotrophin-4 (NT4), ciliary neurotrophic factor (CNTF), pleiotrophin, epidermal growth factor (EGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor type 1 (IGF-1). Yet other examples of neurotrophic agents include agonists and antagonists of these neurotrophic factors or their respective receptors. Examples of agonist and/or antagonists include antibodies against a neurotrophic factor or their receptors and soluble forms of the receptors such as GFR-α (receptor for neurturin); RETα4 (receptor for persephin); GFRα3 (receptor for artemin), TrkA (receptor for NGF), TrkB (receptor for BDNF), TrkC (receptor for NT-3), gp130/LIFRβ (receptor for LIF), and gp130 (receptor for IL6).

[0042] In some embodiments, the compound or composition to be evaluated is a drug or drug candidates for treating neuropathies and include neurotrophic agents as described herein. Examples of drugs that are currently used for the treatment of neuropathic pain, and therefore may be evaluated for neurotrophic activity, include antidepressants (amitriptyline, maprotiline, selective serotonin reuptake inhibitors),

antiepileptics (gabapentin, carbamazepine, clonazepam, lamotrigine, topiramate, phenytoin), local anesthetics, mexiletine, baclofen, clonidine, ketamine, dextrorphan, tramadol, guanethidine, and opioids (morphine, methadone, ketobemidone, fentanyl).

Biomarkers of neurotrophic agents

[0043] The invention provides a method of identifying a biomarker of biological activity of a "neurotrophic agent" (as described herein). The method comprises:

(a) administering the agent to a mammal;

(b) obtaining at least one skin biopsy sample from the mammal;

(c) preparing a tissue extract from the skin biopsy sample; and

(d) determining an amount of at least one nucleic acid or protein in the tissue extract;

wherein a difference in the amount of the nucleic acid or protein determined in step (d) and the amount of the same nucleic acid or protein expressed in the absence of the agent indicates that the nucleic acid or the protein is a biomarker of in vivo biological activity of the agent. In some embodiments, the amount determined in step (d) will differ from the amount of the same nucleic acid or protein expressed in the absence of the agent by, for example, 2, 3, 4, 5, 8, 10, 20, 30, 40, 50, 80, 100-fold, or more. The difference in the levels of expression that is attributed to the presence of biologically active neurotrophic agent is termed "biomarker-fold-change." The greater the biomarker-fold-change value is, the more preferable the nucleic acid or protein is as a biomarker of biological activity of a neurotrophic agent. Some biomarkers (e.g., SEQ ID NO:1120 and SEQ ID NO:1126) may also represent surrogate markers of pain, i.e.,

they correlate with both neuropathic pain and the presence of a biologically active neurotrophic agent. Additionally, some of these biomarkers (e.g., SEQ ID NO:1120 and SEQ ID NO:1126) may also serve as surrogate markers of neurotrophic activity.

[0044] The amount of the same nucleic acid or protein expressed in the absence of the compound or composition can be determined by any suitable method. The skin biopsy sample(s) can be obtained from the same mammal or from different mammals.

[0045] Illustrative methods of identifying a biomarker of biological activity of a "neurotrophic agent" in rats are provided in the Examples below. 49 rat nucleic acids (Table 10) were identified following these methods. Corresponding protein sequences and human orthologues were then identified using publicly available databases such as GenBank™. 15 rat protein sequences (Table 11), 29 human nucleic acid sequences (Table 12); and 29 human protein sequences (Table 13) were identified in this manner.

[0046] The invention provides a method of evaluating biological activity of a neurotrophic agent, comprising:

(a) administering the agent to a mammal;

(b) obtaining at least one skin biopsy sample from the mammal;

(c) preparing a tissue extract from the skin biopsy sample; and

(d) determining an amount of at least one nucleic acid or protein in the tissue extract; the nucleic acid or protein being a biomarker of the biological activity of the neurotrophic agent;

wherein a difference in the amount of the nucleic acid or protein determined in step (d)

and the amount of the same nucleic acid or protein expressed in the absence of the agent indicates that the agent is biologically active. In some embodiments, the amount determined in step (d) will differ from the amount of the same nucleic acid or protein expressed in the absence of the agent by, for example, 2, 3, 4, 5, 8, 10, 20, 30, 40, 50, 80, 100-fold, or more.

[0047] The amount of the same nucleic acid or protein expressed in the absence of the compound or composition can be determined by any suitable method. The skin biopsy sample(s) can be obtained from the same mammal or from different mammals.

[0048] In illustrative embodiments, the neurotrophic agent being evaluated is artemin, a member of the GDNF family.

[0049] In some embodiments, the biomarker of biological activity of a neurotrophic agent is a nucleic acid. In illustrative embodiments, the nucleic acid comprises a nonredundant subsequence of any one of the rat nucleotide sequences of SEQ ID NOs:1115-1163, preferably SEQ ID NOs:1115-1120. In other illustrative embodiments, a biomarker of biological activity of a neurotrophic agent is a nucleic acid that comprises a nonredundant subsequence of any one of the human nucleotide sequences of SEQ ID NOs:1179-1207, preferably SEQ ID NOs:1179-1182.

[0050] In some embodiments, the biomarker of biological activity of a neurotrophic agent is a protein. In illustrative embodiments, the protein comprises a nonredundant subsequence of any one of the rat protein sequences of SEQ ID NOs:1164-1178, preferably SEQ ID NOs:1164-1166. In other illustrative embodiments, a biomarker of biological activity of a neurotrophic agent is a protein

that comprises a nonredundant subsequence of any one of the human protein sequences of SEQ ID NOs:1208-1236, preferably SEQ ID NOs:1208-1211.

General Methods

[0051] Various methods for obtaining skin biopsies are available. The least invasive is removal of the epidermis by placing a suction capsule with over the skin for 30-90 min to develop the blister. The epidermis separates cleanly at the dermal-epidermal junction (Kennedy et al. (1999) Muscle Nerve, 98:323-329; United States Patent No. 6,071,247). This approach is painless and occurs without bleeding because all of the blood vessels terminate beneath the epidermis in the dermal papillae. For these reasons it may be particularly safe on, for example, the feet of diabetic patients. Another approach is simple punch biopsy of the skin. This procedure is also well tolerated. If the biopsy diameter is restricted to 3 mm or less no suture is needed. The biopsy site heals by granulation and leaves a small circular scar that gradually resolves.

[0052] Expression levels, at the RNA or at the protein level, can be determined using conventional methods. Expression levels are usually scaled and/or normalized per total amount of RNA or protein in the sample and/or a control, which is typically a housekeeping gene such actin or GAPDH). RNA levels may be determined by, e.g., quantitative PCR (e.g., TaqMan™ PCR or RT-PCR), Northern blotting, or any other method for determining RNA levels, e.g., as described in Sambrook et al. (eds.) Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, 1989, or Lodie et al. (2002) Tissue Eng., 8(5):739-751), or as described in the Examples. Protein levels may be determined, .e.g., by using Western blotting, ELISA, enzymatic

activity assays, or any other method for determining protein levels, e.g., as described in Current Protocols in Molecular Biology (Ausubel et al. (eds.) New York: John Wiley and Sons, 1998).

[0053] One or more markers of the same or different type can be used in the in the methods of the invention. For example, 1, 2, 3, 4, 5 or more nucleic acids and/or 1, 2, 3, 4, 5 or more proteins can be used for a read-out for (a) neuropathic pain, (b) effect of a compound or composition on the level of neuropathic pain, and/or (c) evaluating biological activity of a neurotrophic agent.

[0054] While representative procedures shown in the Examples are performed using rodents, a skilled artisan will recognize that such procedures can be successfully performed in other mammal and within parameters clinically feasible in human subjects. For example, skin biopsies can be obtained from human patients having neuropathic pain and then subjected to a similar analysis as described herein. For human samples, commercially or custom-made human gene arrays can be used (e.g., Affymatrix™ Human Genome sets U133, U133A, and U95).

[0055] The term "nonredundant subsequence," as used herein, refers to a subsequence which is unique to the sequence in which it occurs. In some embodiments, a nonredunant subsequence is at least, for example, 10, 15, 20, 30, 40, 50, 70, 100, 200, 300, 400, 500, 1000, or 1500 nucleotides long.

[0056] All or some of the following sequences and their nonredundant subsequences can be excluded from certain embodiments: (a) rat DNA SMPs as set out in SEQ ID NOs: 8, 15, 100, 171, 199, 244; (b) rat protein SMPs as set out in SEQ ID NOs: 315, 318, 408, 420; (c) human DNA SMPs as set out in SEQ ID NOs: 476,

478, 568, 578; (d) human protein SMP sas set out in SEQ ID NOs: 636, 638, 728, 738; (e) rat DNA SMNs as set out in SEQ ID NOs: 798, 834; (f) rat protein SMN set out in SEQ ID NO:903; (g) human DNA SMN set out in SEQ ID NO:967; (h) human protein SMN as set out in SEQ ID NO:1043; and (i) sequences disclosed U.S. Patent Application Publication No. US2003/0216341.

Table 2. Rat DNA SMPs

SEQ ID NO:	AffyID™	Accession Number	SEQ ID NO:	AffyID™	Accession Number
1	rc_AI639444_at	NM_057191*	26	X74832cds_at	NM_024485*
2	rc_AA946094_at	NM_021588	27	rc_AI105049_at	AI105049
3	rc_AA891522_f_at	NM_017240*	28	rc_AA866452_s_at	AA866452*
4	rc_AA799471_at	AA799471	29	AA108284_at	NM_019292*
5	rc_AI172339_at	NM_175844	30	rc_AI104913_at	NM_013044*
6	U31816_s_at	U31816	31	rc_AA818120_at	AA818120
7	M24393_at	NM_017115*	32	X59864mRNA_at	X59864
8	M98819mRNA_s_at	M98819	33	AF039832_g_at	NM_019334
9	rc_AI010701_at	AI010701	34	X15939_i_at	NM_017240*
10	rc_AI010736_at	AI010736	35	rc_AA851497_f_at	AA851497
11	X07314cds_at	X07314*	36	rc_AA818845_at	AA818845
12	rc_AI639444_g_at	NM_057191*	37	X14812_at	NM_012606*
13	rc_AA799396_at	AA799396	38	rc_AA800206_at	AA800206*
14	rc_AI169831_at	AI169831	39	X15939_r_at	NM_017240*
15	rc_AI012182_s_at	NM_033234	40	rc_AA901245_at	AA901245
16	L04684_at	L04684	41	X59864mRNA_g_at	X59864
17	rc_AI171653_at	AI171653	42	rc_AA924417_f_at	NM_053395*
18	rc_AA818804_at	AA818804	43	rc_AI170763_at	AI170763
19	rc_AI044544_at	AI044544	44	rc_AI170764_at	AI170764
20	M12098_s_at	NM_012604*	45	rc_AA818947_at	AA818947
21	rc_AI073178_at	AI073178	46	X81193_at	NM_057144*
22	M27151_at	NM_013172*	47	rc_AI170760_at	AI170760
23	rc_AI227690_at	AI227690	48	X80130cds_i_at	X80130
24	rc_AI175100_at	AI175100	49	rc_AA818952_at	AA818952
25	AF077338_at	NM_031813*	50	rc_AA819140_at	NM_019292*

Table 2 (continued).

SEQ ID NO:	AffyID™	Accession Number	SEQ ID NO:	AffyID™	Accession Number
51	rc_AI170696_at	NM_133583	76	rc_AA894101_g_at	AA894101
52	rc_AI170687_at	AI170687	77	rc_AA892468_at	NM_138836
53	rc_AA998888_f_at	AA998888*	78	rc_AI233870_at	AI233870
54	rc_AA819891_at	AA819891	79	X96437mRNA_g_at	X96437
55	rc_AA998685_f_at	AA998685*	80	Y09453cds_at	NM_019255
56	rc_AA998374_f_at	AA998374	81	Z78279_at	Z78279
57	rc_AA849917_at	AA849917	82	rc_AI172054_at	AI172054
58	rc_AA819868_at	AA819868	83	rc_AI172150_at	AI172150
59	rc_AA849501_s_at	AA849501*	84	rc_AI172171_at	AI172171
60	rc_AA819699_at	AA819699	85	rc_AA875206_at	NM_053747
61	rc_AA892801_at	NM_017245	86	rc_AI172189_at	AI172189
62	rc_AA875288_at	AA875288	87	rc_AA859931_g_at	AA859931
63	rc_AA891037_at	AA891037	88	rc_AI233915_at	AI233915
64	rc_AA891903_at	AA891903	89	rc_AI236229_at	AI236229
65	rc_AA891938_at	AA891938	90	rc_AA849974_at	AA849974
66	rc_AA892287_at	AA892287	91	rc_AA858869_at	AA858869
67	rc_AA892313_at	AA892313	92	rc_AA858921_at	AA858921
68	rc_AI172259_at	AI172259	93	rc_AA859335_at	NM_017147*
69	rc_AA892468_g_at	NM_138836	94	X90475cds_at	X90475*
70	rc_AA859829_g_at	AA859829	95	rc_AI172183_at	AI172183
71	rc_AA892860_g_at	AA892860	96	K03467_s_at	NM_012604*
72	rc_AA892999_at	AA892999	97	H32169_at	H32169
73	rc_AA893195_at	AA893195	98	H32451_at	H32451
74	rc_AA893199_at	AA893199	99	J00692_at	J00692*
75	rc_AA893307_at	AA893307	100	J01435cds#8_s_at	J01435

Table 2 (continued).

SEQ ID NO:	AffyID™	Accession Number	SEQ ID NO:	AffyID™	Accession Number
101	J01436cds_s_at	J01436	126	AF086624_s_at	NM_022602
102	J04993_at	NM_017184*	127	AF093536_at	NM_031810
103	L11694_at	NM_017033	128	D37920_at	NM_017136
104	K02423cds_s_at	NM_020104*	129	L13606_at	L13606*
105	D38056_at	NM_053599	130	AF030089UTR#1_at	NM_021584
106	L00088exp_cds#2_at	NM_020104*	131	rc_AA800245_at	AA800245*
107	L00382cds_at	L00382*	132	M83298_g_at	NM_053999
108	L01702_at	NM_012763	133	M83676_at	M83676
109	L01793_at	NM_031043	134	M84176_at	NM_176079*
110	L01793_g_at	NM_031043	135	M86621_at	NM_012919
111	rc_AA799773_g_at	AA799773	136	M89945mRNA_g_at	M89945
112	K02111_at	K02111*	137	rc_AA799571_at	AA799571
113	AF052540_s_at	NM_017117	138	L08505_at	NM_019226
114	AA942808_at	AA942808	139	rc_AA800221_at	NM_053395*
115	AB000216_at	NM_134403	140	M57263_at	NM_031659
116	AB009999_g_at	NM_031242	141	rc_AA800637_at	AA800637
117	AF002281_at	NM_053650	142	rc_AA817802_at	AA817802
118	AF008439_at	NM_013173	143	rc_AA817929_at	AA817929
119	AF013144_at	NM_133578	144	rc_AA817969_at	AA817969
120	E12625cds_at	NM_080886	145	rc_AA817975_at	NM_031355
121	AF037072_at	NM_019292*	146	rc_AA818745_at	AA818745
122	D64046_at	NM_022185	147	rc_AA799773_at	AA799773
123	AF061726_s_at	NM_017117*	148	M21759mRNA_at	M21759
124	AF077338_g_at	NM_031813*	149	L24897_s_at	L24897*
125	AF080507_at	AF080507	150	L27124_s_at	NM_012993

Table 2 (continued).

SEQ ID NO:	AffyID™	Accession Number	SEQ ID NO:	AffyID™	Accession Number
151	L28818cds_at	NM_022195	176	rc_AI236301_at	AI236301
152	M10140_at	NM_012530*	177	rc_AI237371_at	NM_031812
153	M13100cds#2_s_at	M13100	178	rc_AI237700_at	AI237700
154	M16112_at	NM_021739	179	rc_AI638960_at	AI638960
155	M63122_at	NM_013091	180	rc_AI638986_s_at	AI638986
156	M18330_at	NM_133307	181	rc_AI171376_at	NM_053395*
157	M62752_at	NM_012660	182	rc_AI231279_at	AI231279
158	M23995_g_at	NM_017272	183	rc_AI175328_at	AI175328
159	M27434_s_at	NM_147214	184	rc_AA945861_at	AA945861
160	M32397_at	NM_020072	185	rc_AI171774_at	AI171774*
161	M37941mRNA_s_at	NM_138876	186	rc_AI172006_at	NM_021666
162	M37942 exon#2-3_s_at	M37942	187	rc_AI172423_at	NM_181368
163	M55534mRNA_s_at	NM_012935	188	rc_AI172597_at	AI172597
164	rc_AA818807_at	AA818807	189	rc_AI175011_at	AI175011
165	M16112_g_at	NM_021739	190	rc_AI179243_at	NM_145775
166	rc_AI232024_f_at	NM_017239*	191	rc_AI175258_at	AI175258
167	rc_AI180281_at	NM_175843	192	rc_AI178921_s_at	NM_013159
168	rc_AI180442_at	NM_031840	193	rc_AI175348_at	AI175348
169	rc_AI227677_at	AI227677	194	rc_AI175507_at	AI175507
170	rc_AI230247_s_at	NM_019192	195	rc_AI175539_at	NM_022499*
171	rc_AI230319_at	NM_171992	196	rc_AI175935_at	NM_173101*
172	rc_AI230596_at	AI230596	197	rc_AI176584_at	NM_012817
173	rc_AI639187_at	AI639187	198	rc_AI178559_at	NM_012923
174	rc_AI231572_at	AI231572	199	rc_AI639233_s_at	AI639233
175	rc_AI178893_at	AI178893	200	rc_AI175045_at	AI175045

Table 2 (continued).

SEQ ID NO:	AffyID™	Accession Number	SEQ ID NO:	AffyID™	Accession Number
201	X70871_at	NM_012923	226	S74265_s_at	NM_013066
202	X52311_at	NM_054006	227	X12554cds_s_at	NM_012812
203	X53504cds_at	X53504	228	U25651_at	NM_031715*
204	X53504cds_g_at	X53504	229	U30938_at	NM_013066
205	X56133_at	X56133*	230	U40836mRNA_s_at	NM_012786
206	X60351cds_s_at	NM_012935	231	U50736_s_at	NM_013220
207	X64401cds_s_at	NM_173144	232	U84727_at	NM_022398
208	rc_AI639178_at	AI639178	233	U96130_at	NM_031043
209	X70369_s_at	X70369	234	rc_AI171372_at	AI171372
210	X04267_at	NM_012604*	235	S49760_at	NM_080787
211	X74835cds_at	NM_019298	236	rc_AI029057_at	AI029057
212	X76489cds_g_at	X76489	237	rc_AI010583_at	NM_133424
213	X78848cds_f_at	NM_031509	238	rc_AI010605_at	AI010605*
214	X80130cds_f_at	X80130*	239	rc_AI010742_at	AI010742
215	Z78279_g_at	Z78279	240	rc_AI011563_s_at	AI011563
216	Z83869cds_at	NM_021699	241	rc_AI011709_at	AI011709
217	X64827cds_s_at	NM_012786*	242	rc_AI011855_at	AI011855
218	U20195_s_at	NM_017033	243	rc_AI070208_at	AI070208
219	rc_AI639324_at	AI639324	244	rc_AI014135_g_at	AI014135
220	rc_AI639410_i_at	AI639410	245	rc_AA996612_at	AA996612
221	rc_AI639410_s_at	AI639410	246	rc_AI029152_at	AI029152
222	rc_AI639465_f_at	NM_080903*	247	rc_AI030091_at	AI030091
223	rc_AI639532_at	AI639532	248	rc_AI043640_at	AI043640
224	rc_H33725_at	NM_138531	249	rc_AI044292_s_at	AI044292
225	X15939_f_at	NM_017240*	250	rc_AI045097_at	AI045097

Table 2 (continued).

SEQ ID NO:	AffyID™	Accession Number	SEQ ID NO:	AffyID™	Accession Number
251	rc_AI171535_s_at	AI171535	276	rc_AI113309_at	AI113309
252	rc_AI014132_at	AI014132	277	rc_AI136540_at	AI136540*
253	rc_AA946469_at	AA946469	278	rc_AA924428_at	AA924428
254	rc_AA924500_at	AA924500	279	rc_AI145367_at	NM_053874
255	rc_AA925122_at	AA925122	280	rc_AI104864_g_at	AI104864
256	rc_AA925342_at	AA925342	281	rc_AI169265_at	AI169265
257	rc_AA925664_at	AA925664	282	rc_AI170777_at	NM_024398
258	rc_AA944401_at	AA944401	283	rc_AI170777_g_at	NM_024398
259	rc_AA944560_at	NM_153469	284	rc_AI170793_at	AI170793
260	rc_AI010562_at	AI010562*	285	rc_AI170894_at	AI170894
261	rc_AA946457_at	AA946457	286	rc_AI170985_at	NM_020104*
262	rc_AA997341_at	NM_053326	287	rc_AI171098_at	AI171098*
263	rc_AA955927_at	AA955927	288	rc_AI137958_at	AI137958
264	rc_AA957123_at	AA957123	289	rc_AI103376_at	AI103376
265	rc_AA963167_at	AA963167	290	rc_AI071299_at	NM_031135
266	rc_AA963627_at	AA963627	291	rc_AI071328_at	AI071328
267	rc_AA963742_at	AA963742	292	rc_AI071769_at	AI071769
268	rc_AA964584_at	AA964584	293	rc_AI072166_at	AI072166
269	rc_AI070399_at	AI070399	294	rc_AI101481_at	AI101481
270	rc_AA946108_at	NM_173306	295	rc_AI111401_s_at	AI111401
271	rc_AI168935_at	AI168935	296	rc_AI102103_g_at	NM_031083
272	rc_AI104924_f_at	NM_017239*	297	rc_AI103473_at	NM_021865
273	rc_AI059955_s_at	NM_053959	298	rc_AI103507_at	AI103507
274	rc_AI112050_at	AI112050	299	rc_AI103920_f_at	NM_017239*
275	rc_AI112084_at	AI112084	300	rc_AI104035_s_at	AI104035

Table 2 (continued).

SEQ ID NO:	AffyID™	Accession Number	SEQ ID NO:	AffyID™	Accession Number
301	rc_AI104326_at	AI104326	305	rc_AI104567_g_at	AI104567*
302	rc_AI104349_at	AI104349	306	rc_AA892861_at	AA892861
303	rc_AI104354_at	AI104354	307	rc_AI179358_at	AI179358
304	rc_AI102057_at	AI102057	308	AA799397_at	AA799397

* Muscle-specific

Table 3. Rat Protein SMPs

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:
309	rc_AI639444_at	Q9ER30*	1	334	X81193_at	P50463*	46
310	rc_AA946094_at	Q9QZ76	2	335	X80130cds_i_at	P04270	48
311	rc_AA891522_f_at	P02564*	3	336	rc_AA819140_at	P14141*	50
312	rc_AI172339_at	Q8K4K7	5	337	rc_AI170696_at	Q8VBU2	51
313	U31816_s_at	Q02485	6	338	rc_AA819891_at	Q9R272	54
314	M24393_at	P20428*	7	339	rc_AA849501_s_at	Q63518*	59
315	M98819 mRNA_s_at	NP_620231	8	340	rc_AA892801_at	P05197	61
316	X07314cds_at	P08733*	11	341	rc_AA892468_g_at	Q9ES87	69
317	rc_AI639444_g_at	Q9ER30*	12	342	rc_AA892468_at	Q9ES87	77
318	rc_AI012182_s_at	P02091	15	343	Y09453cds_at	P97707	80
319	L04684_at	Q02485	16	344	Z78279_at	Q63079	81
320	M12098_s_at	Q9QZV8*	20	345	rc_AA875206_at	Q9JJP9	85
321	M27151_at	P19335*	22	346	rc_AA858869_at	AAO34127	91
322	AF077338_at	O88599*	25	347	rc_AA859335_at	P45592*	93
323	X74832cds_at	P25108*	26	348	X90475cds_at	Q63518*	94
324	rc_AA866452_s_at	P03996*	28	349	K03467_s_at	Q9QZV8*	96
325	AA108284_at	P14141*	29	350	J00692_at	P02568*	99
326	rc_AI104913_at	P70567*	30	351	J01436cds_s_at	AAA99907	101
327	X59864mRNA_at	Q03668**	32	352	J04993_at	P13413*	102
328	AF039832_g_at	Q9R0W1	33	353	L11694_at	P38652	103
329	X15939_i_at	P02564*	34	354	K02423cds_s_at	NP_064489*	104
330	X14812_at	P16409*	37	355	D38056_at	P97553	105
331	X15939_r_at	P02564*	39	356	L00088exp_cds#	NP_064489*	106
332	X59864 mRNA_g_at	Q03668**	41	357	L00382cds_at	AAA42289*	107
333	rc_AA924417_f_at	Q925F0*	42	358	L01702_at	Q03348	108

Table 3 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:
359	L01793_at	O08730	109	384	rc_AA800221_at	Q925F0*	139
360	L01793_g_at	O08730	110	385	M57263_at	P23606	140
361	K02111_at	P04462*	112	386	rc_AA817975_at	Q9R1Z0	145
362	AF052540_s_at	P16259	113	387	M21759mRNA_at	Q99053	148
363	AB000216_at	O08764	115	388	L24897_s_at	Q63350*	149
364	AB009999_g_at	O35052	116	389	L27124_s_at	25499	150
365	AF002281_at	O70208	117	390	L28818cds_at	NP_071531	151
366	AF008439_at	O54902	118	391	M10140_at	P00564*	152
367	AF013144_at	O54838	119	392	M16112_at	Q63094	154
368	E12625cds_at	O35532	120	393	M63122_at	P22934	155
369	AF037072_at	P14141*	121	394	M18330_at	170538	156
370	D64046_at	Q63788	122	395	M62752_at	P27706	157
371	AF061726_s_at	P16259*	123	396	M23995_g_at	P13601	158
372	AF077338_g_at	O88599*	124	397	M27434_s_at	P02761	159
373	AF086624_s_at	O70444	126	398	M32397_at	P20646	160
374	AF093536_at	O89117	127	399	M37941mRNA_s_at	P10759	161
375	D37920_at	P52020	128	400	M37942exn#2-3	NP_620231	162
376	L13606_at	Q07443*	129	401	M55534mRNA_s_at	P23928	163
377	AF030089UTR#1_at	Q9WVP7	130	402	M16112_g_at	Q63094	165
378	M83298_g_at	P36876	132	403	rc_AI232024_f_at	P02563*	166
379	M83676_at	P35284	133	404	rc_AI180281_at	O08623	167
380	M84176_at	NP_788268*	134	405	rc_AI180442_at	P05369	168
381	M86621_at	P54290	135	406	rc_AI227677_at	Q62940	169
382	M89945mRNA_g_at	NP_114028	136	407	rc_AI230247_s_at	P25236	170
383	L08505_at	P38650	138	408	rc_AI230319_at	P39948	171

Table 3 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:
409	rc_AI231572_at	AAP29778	174	434	Z78279_g_at	Q63079	215
410	rc_AI237371_at	Q9QX82	177	435	Z83869cds_at	O08679	216
411	rc_AI171376_at	Q925F0*	181	436	X64827cds_s_at	P16221*	217
412	rc_AI172006_at	Q9QX75	186	437	U20195_s_at	P38652	218
413	rc_AI172423_at	AAP12535	187	438	rc_AI639465_f_at	Q91Z63*	222
414	rc_AI179243_at	Q63503	190	439	rc_H33725_at	Q8R424	224
415	rc_AI178921_s_at	P35559	192	440	X15939_f_at	P02564*	225
416	rc_AI175539_at	P02625*	195	441	S74265_s_at	P15146	226
417	rc_AI175935_at	Q63356*	196	442	X12554cds_s_at	P10817	227
418	rc_AI176584_at	P24594	197	443	U25651_at	P47858*	228
419	rc_AI178559_at	P39950	198	444	U30938_at	P15146	229
420	rc_AI639233_s_at	Q01129	199	445	U40836mRNA_s_at	P16221	230
421	X70871_at	P39950	201	446	U50736_s_at	Q8R560	231
422	X52311_at	P18395	202	447	U84727_at	P97700	232
423	X53504cds_at	P23358	203	448	U96130_at	O08730	233
424	X53504cds_g_at	P23358	204	449	S49760_at	140866	235
425	X56133_at	P15999*	205	450	rc_AI010583_at	Q8R4I6	237
426	X60351cds_s_at	P23928	206	451	rc_AA996612_at	Q9Z2J4	245
427	X64401cds_s_at	P04800	207	452	rc_AA946469_at	AAP29778	253
428	X70369_s_at	P13941	209	453	rc_AA925664_at	O08813	257
429	X04267_at	Q9QZV8*	210	454	rc_AA944560_at	AAN15275	259
430	X74835cds_at	P25110	211	455	rc_AI010562_at	Q63350*	260
431	X76489cds_g_at	P40241	212	456	rc_AA997341_at	Q62920	262
432	X78848cds_f_at	Q9JLX3	213	457	rc_AA946108_at	P70570	270
433	X80130cds_f_at	P04270*	214	458	rc_AI104924_f_at	P02563*	272

Table 3 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:
459	rc_AI059955_s_at	O08839	273	465	rc_AI071299_at	O08876	290
460	rc_AI145367_at	P52481	279	466	rc_AI111401_s_at	O35217	295
461	rc_AI170777_at	Q9ER34	282	467	rc_AI102103_g_at	O08561	296
462	rc_AI170777_g_at	Q9ER34	283	468	rc_AI103473_at	Q925T0	297
463	rc_AI170985_at	NP_064489*	286	469	rc_AI103920_f_at	P02563*	299
464	rc_AI171098_at	Q63518*	287	470	rc_AI104567_g_at	P03996*	305

* Muscle-specific

** SPTREMBL

Table 4. Human DNA SMPs

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:
471	rc_AI639444_at	NM_006063.1*	1	496	rc_AA819140_at	NM_005181.2*	50
472	rc_AA946094_at	NM_005368.1	2	497	rc_AI170696_at	NM_016250.1	51
473	rc_AA891522_f_at	NM_000257.1*	3	498	rc_AA892801_at	NM_001961.2	61
474	rc_AA799471_at	2330600	4	499	rc_AA892287_at	NM_018653	66
475	M24393_at	NM_002479.2*	7	500	rc_AA892313_at	NM_003193	67
476	M98819mRNA_s_at	NM_000036	8	501	rc_AA892468_g_at	NM_002773.2	69
477	rc_AI639444_g_at	NM_006063.1*	12	502	rc_AA859829_g_at	NM_005882	70
478	rc_AI012182_s_at	NM_000518.4	15	503	rc_AA892468_at	NM_002773.2	77
479	L04684_at	NM_000719	16	504	X96437mRNA_g_at	NM_002727	79
480	rc_AA818804_at	7022045	18	505	Y09453cds_at	NM_000727.2	80
481	M12098_s_at	NM_002470.1*	20	506	Z78279_at	BC036531	81
482	M27151_at	NM_002469.1*	22	507	rc_AA875206_at	222989_s_at	85
483	AF077338_at	NM_004997.1*	25	508	rc_AA859931_g_at	NM_024069	87
484	X74832cds_at	NM_000079.1*	26	509	rc_AA859335_at	NM_005507.1*	93
485	rc_AA866452_s_at	BC009978*	28	510	K03467_s_at	NM_002470.1*	96
486	AA108284_at	NM_005181.2*	29	511	H32169_at	BC018256	97
487	rc_AI104913_at	NM_003275.1*	30	512	J00692_at	NM_009606*	99
488	rc_AA818120_at	1943766	31	513	J04993_at	NM_003281.2*	102
489	AF039832_g_at	NM_000325.3	33	514	L11694_at	NM_002633.2	103
490	X15939_i_at	NM_000257.1*	34	515	K02423cds_s_at	NM_079420.1*	104
491	X14812_at	NM_000258.1*	37	516	D38056_at	NM_004428.2	105
492	X15939_r_at	NM_000257.1*	39	517	L00088exp_cds#2_at	NM_079420.1*	106
493	rc_AA924417_f_at	NM_014332.1*	42	518	L00382cds_at	X06825*	107
494	X81193_at	NM_003476.1*	46	519	L01702_at	NM_002836.2	108
495	X80130cds_i_at	BC009978	48	520	L01793_at	NM_004130.2	109

Table 4 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:
521	L01793_g_at	NM_004130.2	110	546	M57263_at	NM_000359.1	140
522	K02111_at	NM_002470*	112	547	rc_AA817975_at	NM_005662.3	145
523	AF052540_s_at	NM_000070.2	113	548	rc_AA818745_at	29488	146
524	AB000216_at	NM_145804.1	115	549	M21759mRNA_at	XM_048104	148
525	AB009999_g_at	NM_001263.2	116	550	L24897_s_at	XM_028522*	149
526	AF002281_at	NM_014476.1	117	551	L27124_s_at	NM_002525.1	150
527	AF008439_at	NM_000617.1	118	552	L28818cds_at	BC046391	151
528	AF013144_at	NM_004419.2	119	553	M10140_at	NM_001824.2*	152
529	E12625cds_at	NM_006745.2	120	554	M16112_at	NM_001220.3	154
530	AF037072_at	NM_005181.2*	121	555	M63122_at	NM_001065.2	155
531	D64046_at	NM_005027.1	122	556	M18330_at	NM_006254.2	156
532	AF061726_s_at	NM_000070.2*	123	557	M62752_at	NM_001958.2	157
533	AF077338_g_at	NM_004997.1*	124	558	M23995_g_at	NM_000692	158
534	AF093536_at	BC047677	127	559	M32397_at	NM_001099.2	160
535	D37920_at	NM_003129.2	128	560	M37941mRNA_s_at	NM_000036.1	161
536	L13606_at	XM_028522*	129	561	M37942exn#2-3_s_at	NM_000036	162
537	AF030089UTR#1_at	NM_004734.1	130	562	M55534mRNA_s_at	NM_001885.1	163
538	M83298_g_at	NM_002717.2	132	563	M16112_g_at	NM_001220.3	165
539	M83676_at	5410327	133	564	rc_AI232024_f_at	NM_002471.1*	166
540	M84176_at	NM_002478*	134	565	rc_AI180442_at	NM_002004.1	168
541	M86621_at	NM_000722.1	135	566	rc_AI227677_at	D42055.1	169
542	M89945mRNA_g_at	NM_004462	136	567	rc_AI230247_s_at	NM_005410.1	170
543	rc_AA799571_at	13491977	137	568	rc_AI230319_at	NM_001758.1	171
544	L08505_at	NM_001376.2	138	569	rc_AI237371_at	NM_006016.3	177
545	rc_AA800221_at	NM_014332.1*	139	570	rc_AI171376_at	NM_014332.1*	181

Table 4 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:
571	rc_AI172006_at	NM_032467	186	596	rc_H33725_at	NM_006463.2	224
572	rc_AI179243_at	NM_021724.1	190	597	X15939_f_at	NM_000257.1*	225
573	rc_AI178921_s_at	NM_004969.1	192	598	S74265_s_at	NM_002374.2	226
574	rc_AI175539_at	NM_002854.1*	195	599	X12554cds_s_at	NM_005205.2	227
575	rc_AI175935_at	NM_004998.1*	196	600	U25651_at	NM_000289.2*	228
576	rc_AI176584_at	NM_000599.1	197	601	U30938_at	NM_002374.2	229
577	rc_AI178559_at	NM_004060.2	198	602	U40836mRNA_s_at	1311703	230
578	rc_AI639233_s_at	NM_001920.2	199	603	U50736_s_at	NM_014391.1	231
579	X70871_at	NM_004060.2	201	604	U84727_at	NM_003562.3	232
580	X52311_at	NM_002524.2	202	605	U96130_at	NM_004130.2	233
581	X56133_at	NM_004046.3*	205	606	S49760_at	BC043292	235
582	X60351cds_s_at	NM_001885.1	206	607	rc_AI010583_at	NM_001104.1	237
583	X64401cds_s_at	BC003642	207	608	rc_AI011709_at	35526	241
584	X70369_s_at	NM_000090.2	209	609	rc_AA996612_at	NM_144573.1	245
585	X04267_at	NM_002470.1*	210	610	rc_AA925122_at	NM_000363	255
586	X74835cds_at	NM_000751.1	211	611	rc_AA925664_at	NM_001664	257
587	X76489cds_g_at	NM_001769.2	212	612	rc_AA944560_at	NM_007066.3	259
588	X78848cds_f_at	NM_000847.3	213	613	rc_AA997341_at	NM_006457.1	262
589	X80130cds_f_at	BC009978*	214	614	rc_AA946108_at	NM_173306	270
590	Z78279_g_at	BC036531	215	615	rc_AI168935_at	NM_018286	271
591	Z83869cds_at	NM_004954.2	216	616	rc_AI104924_f_at	NM_002471.1*	272
592	X64827cds_s_at	J04823*	217	617	rc_AI059955_s_at	NM_004305.2	273
593	U20195_s_at	NM_002633.2	218	618	rc_AI145367_at	NM_006366.1	279
594	rc_AI639324_at	NM_030793	219	619	rc_AI170777_at	NM_001098.1	282
595	rc_AI639465_f_at	NM_032588.2*	222	620	rc_AI170777_g_at	NM_001098.1	283

Table 4 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:
621	rc_AI170894_at	NM_001122	288	626	rc_AI102103_g_at	NM_002651.1	278
622	rc_AI170985_at	NM_079420.1*	300	627	rc_AI103473_at	NM_018664.1	237
623	rc_AI103376_at	NM_018112	282	628	rc_AI103920_f_at	NM_002471.1*	281
624	rc_AI071299_at	NM_005655.1	235	629	rc_AI104354_at	NM_016599	93
625	rc_AI111401_s_at	NM_004897.2	289	630	rc_AI104567_g_at	BC009978*	200

* Muscle-specific

Table 5. Human Protein SMPs

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	Table 4 SEQ ID NO:
631	rc_AI639444_at	AAH06534*	1	471
632	rc_AA946094_at	P02144	2	472
633	rc_AA891522_f_at	P12883*	3	473
634	rc_AA799471_at	O15273	4	474
635	M24393_at	P15173*	7	475
636	M98819mRNA_s_at	NP_000027	8	476
637	rc_AI639444_g_at	AAH06534*	12	477
638	rc_AI012182_s_at	P02023	15	478
639	L04684_at	NP_000710	16	479
640	rc_AA818804_at	Q9NWB1	18	480
641	M12098_s_at	P11055*	20	481
642	M27151_at	AAH17834*	22	482
643	AF077338_at	AAH44226*	25	483
644	X74832cds_at	P02708*	26	484
645	rc_AA866452_s_at	AAH009987*	28	485
646	AA108284_at	P07451*	29	486
647	rc_AI104913_at	P28289*	30	487
648	rc_AA818120_at	O00631	31	488
649	AF039832_g_at	Q99697	33	489
650	X15939_i_at	P12883*	34	490
651	X14812_at	AAH09790*	37	491
652	X15939_r_at	P12883*	39	492
653	rc_AA924417_f_at	Q9UHP9*	42	493
654	X81193_at	P50461*	46	494
655	X80130cds_i_at	AAH009987	48	495

Table 5 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	Table 4 SEQ ID NO:
656	rc_AA819140_at	P07451*	50	496
657	rc_AI170696_at	CAD62321	51	497
658	rc_AA892801_at	P13639	61	498
659	rc_AA892287_at	NP_061123.2	66	499
660	rc_AA892313_at	NP_003184.1	67	500
661	rc_AA892468_g_at	Q16651	69	501
662	rc_AA859829_g_at	NP_005873.1	70	502
663	rc_AA892468_at	Q16651	77	503
664	X96437mRNA_g_at	AAH22313	79	504
665	Y09453cds_at	Q06432	80	505
666	Z78279_at	P02452	81	506
667	rc_AA875206_at	NP_038466.2	85	507
668	rc_AA859931_g_at	NP_076974.1	87	508
669	rc_AA859335_at	P23528*	93	509
670	K03467_s_at	P11055*	96	510
671	H32169_at	NP_068733.1	97	511
672	J00692_at	NP_033736*	99	512
673	J04993_at	AAH12600*	102	513
674	L11694_at	AAH19920	103	514
675	K02423cds_s_at	AAH05318*	104	515
676	D38056_at	P20827	105	516
677	L00088expanded_cds#2_at	AAH05318*	106	517
678	L00382cds_at	CAA29971*	107	518
679	L01702_at	AAH27308	108	519
680	L01793_at	P46976	109	520

Table 5 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	Table 4 SEQ ID NO:
681	L01793_g_at	P46976	110	521
682	K02111_at	NP_002461*	112	522
683	AF052540_s_at	P20807	113	523
684	AB000216_at	Q8N961	115	524
685	AB009999_g_at	Q92903	116	525
686	AF002281_at	O43590	117	526
687	AF008439_at	BAB93467	118	527
688	AF013144_at	Q16690	119	528
689	E12625cds_at	Q15800	120	529
690	AF037072_at	P07451*	121	530
691	D64046_at	O00459	122	531
692	AF061726_s_at	P20807*	123	532
693	AF077338_g_at	AAH44226*	124	533
694	AF093536_at	AAH47677	127	534
695	D37920_at	Q14534	128	535
696	L13606_at	XP_028522*	129	536
697	AF030089UTR#1_at	O15075	130	537
698	M83298_g_at	AAH41071	132	538
699	M83676_at	O88386	133	539
700	M84176_at	NP_002469*	134	540
701	M86621_at	P54289	135	541
702	M89945mRNA_g_at	NP_004453	136	542
703	rc_AA799571_at	Q9BXS4	137	543
704	L08505_at	BAA20783	138	544
705	rc_AA800221_at	Q9UHP9*	139	545

Table 5 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	Table 4 SEQ ID NO:
706	M57263_at	AAH34699	140	546
707	rc_AA817975_at	Q9Y277	145	547
708	rc_AA818745_at	Q01484	146	548
709	M21759mRNA_at	XP_048104	148	549
710	L24897_s_at	XP_028522*	149	550
711	L27124_s_at	O43847	150	551
712	L28818cds_at	AAH46391	151	552
713	M10140_at	P06732*	152	553
714	M16112_at	AAH19070	154	554
715	M63122_at	P19438	155	555
716	M18330_at	AAH43350	156	556
717	M62752_at	Q05639	157	557
718	M23995_g_at	NP_000683	158	558
719	M32397_at	P15309	160	559
720	M37941mRNA_s_at	P23109	161	560
721	M37942exon#2-3_s_at	NP_000027	162	561
722	M55534mRNA_s_at	P02511	163	562
723	M16112_g_at	AAH19070	165	563
724	rc_AI232024_f_at	O60661*	166	564
725	rc_AI180442_at	P14324	168	565
726	rc_AI227677_at	P46934	169	566
727	rc_AI230247_s_at	P49908	170	567
728	rc_AI230319_at	AAH23620	171	568
729	rc_AI237371_at	O95413	177	569
730	rc_AI171376_at	Q9UHP9*	181	570

Table 5 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	Table 4 SEQ ID NO:
731	rc_AI172006_at	NP_115856	186	571
732	rc_AI179243_at	AAA52334	190	572
733	rc_AI178921_s_at	P14735	192	573
734	rc_AI175539_at	P20472*	195	574
735	rc_AI175935_at	Q12965*	196	575
736	rc_AI176584_at	P24593	197	576
737	rc_AI178559_at	P51959	198	577
738	rc_AI639233_s_at	P07585	199	578
739	X70871_at	P51959	201	579
740	X52311_at	AAH32446	202	580
741	X56133_at	P25705*	205	581
742	X60351cds_s_at	P02511	206	582
743	X64401cds_s_at	AAH03642	207	583
744	X70369_s_at	AAB59383	209	584
745	X04267_at	P11055*	210	585
746	X74835cds_at	Q07001	211	586
747	X76489cds_g_at	P21926	212	587
748	X78848cds_f_at	Q16772	213	588
749	X80130cds_f_at	AAH009987*	214	589
750	Z78279_g_at	P02452	215	590
751	Z83869cds_at	Q15449	216	591
752	X64827cds_s_at	P10176*	217	592
753	U20195_s_at	AAH19920	218	593
754	rc_AI639324_at	NP_110420.1	219	594
755	rc_AI639465_f_at	Q969Q1*	222	595

Table 5 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	Table 4 SEQ ID NO:
756	rc_H33725_at	O95630	224	596
757	X15939_f_at	P12883*	225	597
758	S74265_s_at	P11137	226	598
759	X12554cds_s_at	AAH29818	227	599
760	U25651_at	AAH12799*	228	600
761	U30938_at	P11137	229	601
762	U40836mRNA_s_at	P10176	230	602
763	U50736_s_at	Q15327	231	603
764	U84727_at	Q02978	232	604
765	U96130_at	P46976	233	605
766	S49760_at	S12969	235	606
767	rc_AI010583_at	Q08043	237	607
768	rc_AI011709_at	Q15155	241	608
769	rc_AA996612_at	Q96DL0	245	609
770	rc_AA925122_at	TPHUCC	255	610
771	rc_AA925664_at	A32342	257	611
772	rc_AA944560_at	Q9Y2B9	259	612
773	rc_AA997341_at	O60705	262	613
774	rc_AA946108_at	A55347	270	614
775	rc_AI168935_at	NP_060756.1	271	615
776	rc_AI104924_f_at	O60661*	272	616
777	rc_AI059955_s_at	CAD28496	273	617
778	rc_AI145367_at	P40123	279	618
779	rc_AI170777_at	Q8TAQ6	282	619
780	rc_AI170777_g_at	Q8TAQ6	283	620

Table 5 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	Table 4 SEQ ID NO:
781	rc_AI170894_at	NP_001113.1	285	621
782	rc_AI170985_at	AAH05318*	286	622
783	rc_AI103376_at	NP_060582.1	289	623
784	rc_AI071299_at	O75411	290	624
785	rc_AI111401_s_at	O95172	295	625
786	rc_AI102103_g_at	O15096	296	626
787	rc_AI103473_at	Q9NR55	297	627
788	rc_AI103920_f_at	O60661*	299	628
789	rc_AI104354_at	NP_057683.1	303	629
790	rc_AI104567_g_at	AAH009987*	305	630

* Muscle-specific

Table 6. Rat DNA SMNs

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:
791	rc_AA946094_at	NM_021588	2	816	rc_AA892801_at	NM_017245	61
792	rc_AA891522_f_at	NM_017240*	3	817	rc_AA875288_at	AA875288	62
793	rc_AA799471_at	AA799471	4	818	rc_AA891938_at	AA891938	65
794	rc_AI172339_at	NM_175844	5	819	rc_AA892287_at	AA892287	66
795	M24393_at	NM_017115*	7	820	rc_AA892313_at	AA892313	67
796	X07314cds_at	X07314*	11	821	rc_AA892468_g_at	NM_138836	69
797	rc_AA799396_at	AA799396	13	822	rc_AA859829_g_at	AA859829	70
798	rc_AI012182_s_at	NM_033234	15	823	rc_AA892860_g_at	AA892860	71
799	rc_AA818804_at	AA818804	18	824	rc_AA893307_at	AA893307	75
800	M12098_s_at	NM_012604*	20	825	rc_AA894101_g_at	AA894101	76
801	M27151_at	NM_013172*	22	826	Z78279_at	Z78279	81
802	rc_AI227690_at	AI227690	23	827	rc_AI172054_at	AI172054	82
803	rc_AI175100_at	AI175100	24	828	rc_AA875206_at	NM_053747	85
804	AF077338_at	NM_031813*	25	829	rc_AI172189_at	AI172189	86
805	X74832cds_at	NM_024485*	26	830	rc_AA859931_g_at	AA859931	87
806	AA108284_at	NM_019292*	29	831	rc_AA858921_at	AA858921	92
807	rc_AI104913_at	NM_013044*	30	832	rc_AA859335_at	NM_017147*	93
808	rc_AA818120_at	AA818120	31	833	H32169_at	H32169	97
809	X59864mRNA_at	X59864	32	834	J01435cds#8_s_at	J01435	100
810	AF039832_g_at	NM_019334	33	835	J01436cds_s_at	J01436	101
811	X15939_i_at	NM_017240*	34	836	J04993_at	NM_017184*	102
812	rc_AA851497_f_at	AA851497	35	837	L11694_at	NM_017033	103
813	X14812_at	NM_012606*	37	838	D38056_at	NM_053599	105
814	X15939_r_at	NM_017240*	39	839	L01702_at	NM_012763	108
815	rc_AI170696_at	NM_133583	51	840	AB009999_g_at	NM_031242	116

Table 6 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:
841	AF008439_at	NM_013173	118	866	X53504cds_g_at	X53504	204
842	AF037072_at	NM_019292*	121	867	X64401cds_s_at	NM_173144	207
843	D64046_at	NM_022185	122	868	X76489cds_g_at	X76489	212
844	AF077338_g_at	NM_031813*	124	869	Z78279_g_at	Z78279	215
845	AF080507_at	AF080507	125	870	Z83869cds_at	NM_021699	216
846	D37920_at	NM_017136	128	871	X64827cds_s_at	NM_012786*	217
847	M83298_g_at	NM_053999	132	872	rc_AI639324_at	AI639324	219
848	M83676_at	M83676	133	873	rc_AI639410_i_at	AI639410	220
849	rc_AA799571_at	AA799571	137	874	rc_AI639465_f_at	NM_080903*	222
850	M57263_at	NM_031659	140	875	rc_H33725_at	NM_138531	224
851	rc_AA817975_at	NM_031355	145	876	X12554cds_s_at	NM_012812	227
852	rc_AA818745_at	AA818745	146	877	U30938_at	NM_013066	229
853	L27124_s_at	NM_012993	150	878	U40836mRNA_s_at	NM_012786	230
854	M18330_at	NM_133307	156	879	S49760_at	NM_080787	235
855	M32397_at	NM_020072	160	880	rc_AI011709_at	AI011709	241
856	rc_AI227677_at	AI227677	169	881	rc_AI043640_at	AI043640	248
857	rc_AI230247_s_at	NM_019192	170	882	rc_AI044292_s_at	AI044292	249
858	rc_AI230596_at	AI230596	172	883	rc_AA925122_at	AA925122	255
859	rc_AI638960_at	AI638960	179	884	rc_AA925664_at	AA925664	257
860	rc_AI171376_at	NM_053395*	181	885	rc_AA946108_at	NM_173306	270
861	rc_AI172423_at	NM_181368	187	886	rc_AI168935_at	AI168935	271
862	rc_AI178921_s_at	NM_013159	192	887	rc_AA924428_at	AA924428	278
863	rc_AI175935_at	NM_173101*	196	888	rc_AI170894_at	AI170894	285
864	rc_AI176584_at	NM_012817	197	889	rc_AI103376_at	AI103376	289
865	X52311_at	NM_054006	202	890	rc_AI071299_at	NM_031135	290

Table 6 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:
891	rc_AI072166_at	AI072166	293	895	rc_AI104354_at	AI104354	303
892	rc_AI111401_s_at	AI111401	295	896	rc_AA892861_at	AA892861	306
893	rc_AI103507_at	AI103507	298	897	rc_AI179358_at	AI179358	307
894	rc_AI104349_at	AI104349	302				

* Muscle-specific

Table 7. Rat Protein SMPs

SEQ ID NO:	AffyID™	Accession Number	Table 6 SEQ ID NO:	Table 3 SEQ ID NO:
898	rc_AA946094_at	Q9QZ76	2	791
899	rc_AA891522_f_at	P02564*	3	792
900	rc_AI172339_at	Q8K4K7	5	794
901	M24393_at	P20428*	7	795
902	X07314cds_at	P08733*	11	796
903	rc_AI012182_s_at	P02091	15	798
904	M12098_s_at	Q9QZV8*	20	800
905	M27151_at	P19335*	22	801
906	AF077338_at	O88599*	25	804
907	X74832cds_at	P25108*	26	805
908	AA108284_at	P14141*	29	806
909	rc_AI104913_at	P70567*	30	807
910	X59864mRNA_at	Q03668**	32	809
911	AF039832_g_at	Q9R0W1	33	810
912	X15939_i_at	P02564*	34	811
913	X14812_at	P16409*	37	813
914	X15939_r_at	P02564*	39	814
915	rc_AI170696_at	Q8VBU2	51	815
916	rc_AA892801_at	P05197	61	816
917	rc_AA892468_g_at	Q9ES87	69	821
918	Z78279_at	Q63079	81	826
919	rc_AA875206_at	Q9JJP9	85	828
920	rc_AA859335_at	P45592*	93	832
921	J01436cds_s_at	AAA99907	101	835
922	J04993_at	P13413*	102	836
923	L11694_at	P38652	103	837
924	D38056_at	P97553	105	838
925	L01702_at	Q03348	108	839
926	AB009999_g_at	O35052	116	840
927	AF008439_at	O54902	118	841
928	AF037072_at	P14141*	121	842

Table 7 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 6 SEQ ID NO:	Table 3 SEQ ID NO:
929	D64046_at	Q63788	122	843
930	AF077338_g_at	O88599*	124	844
931	D37920_at	P52020	128	846
932	M83298_g_at	P36876	132	847
933	M83676_at	P35284	133	848
934	M57263_at	P23606	140	850
935	rc_AA817975_at	Q9R1Z0	145	851
936	L27124_s_at	25499	150	853
937	M18330_at	170538	156	854
938	M32397_at	P20646	160	855
939	rc_AI227677_at	Q62940	169	856
940	rc_AI230247_s_at	P25236	170	857
941	rc_AI171376_at	Q925F0*	181	860
942	rc_AI172423_at	AAP12535	187	861
943	rc_AI178921_s_at	P35559	192	862
944	rc_AI175935_at	Q63356*	196	863
945	rc_AI176584_at	P24594	197	864
946	X52311_at	P18395	202	865
947	X53504cds_g_at	P23358	204	866
948	X64401cds_s_at	P04800	207	867
949	X76489cds_g_at	P40241	212	868
950	Z78279_g_at	Q63079	215	869
951	Z83869cds_at	O08679	216	870
952	X64827cds_s_at	P16221*	217	871
953	rc_AI639465_f_at	Q91Z63*	222	874
954	rc_H33725_at	Q8R424	224	875
955	X12554cds_s_at	P10817	227	876
956	U30938_at	P15146	229	877
957	U40836mRNA_s_at	P16221	230	878
958	S49760_at	140866	235	879
959	rc_AA925664_at	O08813	257	884
960	rc_AA946108_at	P70570	270	885
961	rc_AI071299_at	O08876	290	890
962	rc_AI111401_s_at	O35217	295	892

* Muscle-specific

** SPTREMBL

Table 8. Human DNA SMNs

SEQ ID NO:	AffyID™	Accession Number	Table 6 SEQ ID NO:	Table 4 SEQ ID NO:
963	rc_AA946094_at	NM_005368.1	2	791
964	rc_AA891522_f_at	NM_000257.1*	3	792
965	rc_AA799471_at	2330600	4	793
966	M24393_at	NM_002479.2*	7	795
967	rc_AI012182_s_at	NM_000518.4	15	798
968	rc_AA818804_at	7022045	18	799
969	M12098_s_at	NM_002470.1*	20	800
970	M27151_at	NM_002469.1*	22	801
971	AF077338_at	NM_004997.1*	25	804
972	X74832cds_at	NM_000079.1*	26	805
973	AA108284_at	NM_005181.2*	29	806
974	rc_AI104913_at	NM_003275.1*	30	807
975	rc_AA818120_at	1943766	31	808
976	AF039832_g_at	NM_000325.3	33	810
977	X15939_i_at	NM_000257.1*	34	811
978	X14812_at	NM_000258.1*	37	813
979	X15939_r_at	NM_000257.1*	39	814
980	rc_AI170696_at	NM_016250.1	51	815
981	rc_AA892801_at	NM_001961.2	61	816
982	rc_AA892287_at	NM_018653	66	819
983	rc_AA892313_at	NM_003193	67	820
984	rc_AA892468_g_at	NM_002773.2	69	821
985	rc_AA859829_g_at	NM_005882	70	822
986	Z78279_at	BC036531	81	826
987	rc_AA875206_at	222989_s_at	85	828
988	rc_AA859931_g_at	NM_024069	87	830
989	rc_AA859335_at	NM_005507.1*	93	832
990	H32169_at	BC018256	97	833
991	J04993_at	NM_003281.2*	102	836
992	L11694_at	NM_002633.2	103	837

Table 8 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 6 SEQ ID NO:	Table 4 SEQ ID NO:
993	D38056_at	NM_004428.2	105	838
994	L01702_at	NM_002836.2	108	839
995	AB009999_g_at	NM_001263.2	116	840
996	AF008439_at	NM_000617.1	118	841
997	AF037072_at	NM_005181.2*	121	842
998	D64046_at	NM_005027.1	122	843
999	AF077338_g_at	NM_004997.1*	124	844
1000	D37920_at	NM_003129.2	128	846
1001	M83298_g_at	NM_002717.2	132	847
1002	M83676_at	5410327	133	848
1003	rc_AA799571_at	13491977	137	849
1004	M57263_at	NM_000359.1	140	850
1005	rc_AA817975_at	NM_005662.3	145	851
1006	rc_AA818745_at	29488	146	852
1007	L27124_s_at	NM_002525.1	150	853
1008	M18330_at	NM_006254.2	156	854
1009	M32397_at	NM_001099.2	160	855
1010	rc_AI227677_at	D42055.1	169	856
1011	rc_AI230247_s_at	NM_005410.1	170	857
1012	rc_AI171376_at	NM_014332.1*	181	860
1013	rc_AI178921_s_at	NM_004969.1	192	862
1014	rc_AI175935_at	NM_004998.1*	196	863
1015	rc_AI176584_at	NM_000599.1	197	864
1016	X52311_at	NM_002524.2	202	865
1017	X64401cds_s_at	BC003642	207	867
1018	X76489cds_g_at	NM_001769.2	212	868
1019	Z78279_g_at	BC036531	215	869
1020	Z83869cds_at	NM_004954.2	216	870
1021	X64827cds_s_at	J04823*	217	871
1022	rc_AI639324_at	NM_030793	219	872

Table 8 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 6 SEQ ID NO:	Table 4 SEQ ID NO:
1023	rc_AI639465_f_at	NM_032588.2*	222	874
1024	rc_H33725_at	NM_006463.2	224	875
1025	X12554cds_s_at	NM_005205.2	227	876
1026	U30938_at	NM_002374.2	229	877
1027	U40836mRNA_s_at	1311703	230	878
1028	S49760_at	BC043292	235	879
1029	rc_AI011709_at	35526	241	880
1030	rc_AA925122_at	NM_000363	255	883
1031	rc_AA925664_at	NM_001664	257	884
1032	rc_AA946108_at	NM_173306	270	885
1033	rc_AI168935_at	NM_018286	271	886
1034	rc_AI170894_at	NM_001122	285	888
1035	rc_AI103376_at	NM_018112	289	889
1036	rc_AI071299_at	NM_005655.1	290	890
1037	rc_AI111401_s_at	NM_004897.2	295	892
1038	rc_AI104354_at	NM_016599	303	895

* Muscle-specific

Table 9. Human Protein SMNs

SEQ ID NO:	AffyID™	Accession Number	Table 8 SEQ ID NO:	Table 5 SEQ ID NO:
1039	rc_AA946094_at	P02144	2	963
1040	rc_AA891522_f_at	P12883*	3	964
1041	rc_AA799471_at	O15273	4	965
1042	M24393_at	P15173*	7	966
1043	rc_AI012182_s_at	P02023	15	967
1044	rc_AA818804_at	Q9NWB1	18	968
1045	M12098_s_at	P11055*	20	969
1046	M27151_at	AAH17834*	22	970
1047	AF077338_at	AAH44226*	25	971
1048	X74832cds_at	P02708*	26	972
1049	AA108284_at	P07451*	29	973
1050	rc_AI104913_at	P28289*	30	974
1051	rc_AA818120_at	O00631	31	975
1052	AF039832_g_at	Q99697	33	976
1053	X15939_i_at	P12883*	34	977
1054	X14812_at	AAH09790*	37	978
1055	X15939_r_at	P12883*	39	979
1056	rc_AI170696_at	CAD62321	51	980
1057	rc_AA892801_at	P13639	61	981
1058	rc_AA892287_at	NP_061123.2	66	982
1059	rc_AA892313_at	NP_003184.1	67	983
1060	rc_AA892468_g_at	Q16651	69	984
1061	rc_AA859829_g_at	NP_005873.1	70	985
1062	Z78279_at	P02452	81	986
1063	rc_AA875206_at	NP_038466.2	85	987
1064	rc_AA859931_g_at	NP_076974.1	87	988
1065	rc_AA859335_at	P23528*	93	989
1066	H32169_at	NP_068733.1	97	990
1067	J04993_at	AAH12600*	102	991
1068	L11694_at	AAH19920	103	992

Table 9 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 8 SEQ ID NO:	Table 5 SEQ ID NO:
1069	D38056_at	P20827	105	993
1070	L01702_at	AAH27308	108	994
1071	AB009999_g_at	Q92903	116	995
1072	AF008439_at	BAB93467	118	996
1073	AF037072_at	P07451*	121	997
1074	D64046_at	O00459	122	998
1075	AF077338_g_at	AAH44226*	124	999
1076	D37920_at	Q14534	128	1000
1077	M83298_g_at	AAH41071	132	1001
1078	M83676_at	O88386	133	1002
1079	rc_AA799571_at	Q9BXS4	137	1003
1080	M57263_at	AAH34699	140	1004
1081	rc_AA817975_at	Q9Y277	145	1005
1082	rc_AA818745_at	Q01484	146	1006
1083	L27124_s_at	O43847	150	1007
1084	M18330_at	AAH43350	156	1008
1085	M32397_at	P15309	160	1009
1086	rc_AI227677_at	P46934	169	1010
1087	rc_AI230247_s_at	P49908	170	1011
1088	rc_AI171376_at	Q9UHP9*	181	1012
1089	rc_AI178921_s_at	P14735	192	1013
1090	rc_AI175935_at	Q12965*	196	1014
1091	rc_AI176584_at	P24593	197	1015
1092	X52311_at	AAH32446	202	1016
1093	X64401cds_s_at	AAH03642	207	1017
1094	X76489cds_g_at	P21926	212	1018
1095	Z78279_g_at	P02452	215	1019
1096	Z83869cds_at	Q15449	216	1020
1097	X64827cds_s_at	P10176*	217	1021
1098	rc_AI639324_at	NP_110420.1	219	1022

Table 9 (continued).

SEQ ID NO:	AffyID™	Accession Number	Table 8 SEQ ID NO:	Table 5 SEQ ID NO:
1099	rc_AI639465_f_at	Q969Q1*	222	1023
1100	rc_H33725_at	O95630	224	1024
1101	X12554cds_s_at	AAH29818	227	1025
1102	U30938_at	P11137	229	1026
1103	U40836mRNA_s_at	P10176	230	1027
1104	S49760_at	S12969	235	1028
1105	rc_AI011709_at	Q15155	241	1029
1106	rc_AA925122_at	TPHUCC	255	1030
1107	rc_AA925664_at	A32342	257	1031
1108	rc_AA946108_at	A55347	270	1032
1109	rc_AI168935_at	NP_060756.1	271	1033
1110	rc_AI170894_at	NP_001113.1	285	1034
1111	rc_AI103376_at	NP_060582.1	289	1035
1112	rc_AI071299_at	O75411	290	1036
1113	rc_AI111401_s_at	O95172	295	1037
1114	rc_AI104354_at	NP_057683.1	303	1038

* Muscle-specific

Table 10. Rat DNA BMNs

SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:	SEQ ID NO:	AffyID™	Accession Number	Table 2 SEQ ID NO:
1115	rc_AI233261_i_at	NM_017305	N/A	1140	S69316_s_at	S69316	N/A
1116	rc_AI012354_at	NM_022647	N/A	1141	rc_AI639155_at	AI639155	N/A
1117	rc_AA955974_at	AA955974	N/A	1142	rc_AI639012_at	AI639012	N/A
1118	rc_AI072712_at	AI072712	N/A	1143	rc_AI237654_at	AI237654	N/A
1119	rc_AI029088_at	AI029088	N/a	1144	rc_AI228696_at	AI228696	N/A
1120	X15939_i_at	NM_017240*	34	1145	rc_AI177055_at	AI177055	N/A
1121	rc_AA850730_at	AA850730	N/A	1146	rc_AI176969_at	AI176969	N/A
1122	rc_AA998245_at	AA998245	N/A	1147	rc_AI102438_at	AI102438	N/A
1123	rc_AI233173_at	NM_138548	N/A	1148	rc_AI072603_at	AI072603	N/A
1124	rc_AI232350_f_at	AI232350	N/A	1149	rc_AI059519_at	AI059519	N/A
1125	rc_AA998683_at	NM_031970	N/A	1150	rc_AA899491_at	AA899491	N/A
1126	rc_AA858921_at	AA858921	92	1151	rc_AI044635_at	AI044635	N/A
1127	rc_AA850705_at	NM_153309	N/A	1152	rc_AA874873_g_at	AA874873	N/A
1128	rc_AA998097_at	AA998097	N/A	1153	rc_AI014091_at	NM_053698	N/A
1129	rc_AA997841_at	NM_133298	N/A	1154	rc_AI013854_at	AI013854	N/A
1130	rc_AA848449_at	AA848449	N/A	1155	rc_AI012937_at	AI012937	N/A
1131	rc_AA819268_at	AA819268	N/A	1156	rc_AI008911_at	AI008911	N/A
1132	rc_AA800268_at	AA800268	N/A	1157	rc_AI007875_at	NM_053435	N/A
1133	M14050_s_at	NM_013083	N/A	1158	rc_AI007672_at	AI007672	N/A
1134	L06804_at	L06804	N/A	1159	rc_AA997726_at	AA997726	N/A
1135	D12769_at	NM_057211	N/A	1160	rc_AA963457_at	AA963457	N/A
1136	AF036335_g_at	AF036335	N/A	1161	rc_AA963171_at	AA963171	N/A
1137	AA801076_at	AA801076	N/A	1162	rc_AA924573_s_at	AA924573	N/A
1138	rc_AA848829_at	AA848829	N/A	1163	rc_AI058912_at	AI058912	N/A
1139	rc_AI030259_at	AI030259	N/A				

* Muscle-specific

Table 11. Rat Protein BMNs

SEQ ID NO :	AffyID™	Accession Number	Table 10 SEQ ID NO:	Table 2 SEQ ID NO:
1164	rc_AI233261_i_at	P48508	1115	N/A
1165	rc_AI012354_at	64647	1116	N/A
1166	X15939_i_at	P02564*	1120	34
1167	rc_AI233173_at	Q05982	1123	N/A
1168	rc_AA998683_at	P42930	1125	N/A
1169	rc_AA850705_at	Q8CFC1	1127	N/A
1170	rc_AA997841_at	Q9QZF6	1129	N/A
1171	M14050_s_at	P06761	1133	N/A
1172	L06804_at	P36198	1134	N/A
1173	D12769_at	Q01713	1135	N/A
1174	AF036335_g_at	O54725	1136	N/A
1175	S69316_s_at	S69316	1140	N/A
1176	rc_AI237654_at	117514	1143	N/A
1177	rc_AI014091_at	Q99MA1	1153	N/A
1178	rc_AI007875_at	Q9ESZ0	1157	N/A

* Muscle-specific

Table 12. Human DNA BMNs

SEQ ID NO:	AffyID™	Accession Number	Table 10 SEQ ID NO:	Table 4 SEQ ID NO:
1179	rc_AI233261_i_at	NM_002061.1	1115	N/A
1180	rc_AI012354_at	NM_022647	1116	N/A
1181	rc_AA955974_at	NM_001851	1117	N/A
1182	X15939_i_at	NM_000257.1*	1120	490
1183	rc_AA850730_at	505095	1121	N/A
1184	rc_AI233173_at	NM_000269.1	1123	N/A
1185	rc_AA998683_at	NM_031970	1125	N/A
1186	rc_AA850705_at	213189_at	1127	N/A
1187	rc_AA998097_at	1000283	1128	N/A
1188	rc_AA997841_at	NM_002510.1	1129	N/A
1189	rc_AA800268_at	NM_014182	1132	N/A
1190	M14050_s_at	NM_005347.2	1133	N/A
1191	L06804_at	600494	1134	N/A
1192	D12769_at	NM_001206.1	1135	N/A
1193	AF036335_g_at	NM_005066.1	1136	N/A
1194	rc_AI030259_at	NM_031219	1139	N/A
1195	rc_AI639012_at	NM_024042	1142	N/A
1196	rc_AI237654_at	NM_006472.1	1143	N/A
1197	rc_AI228696_at	NM_012106	1144	N/A
1198	rc_AI177055_at	NM_053050	1145	N/A
1199	rc_AI059519_at	NM_024324	1149	N/A
1200	rc_AA899491_at	XM_291885	1150	N/A
1201	rc_AI044635_at	NM_014934	1151	N/A
1202	rc_AI014091_at	NM_006079.2	1153	N/A
1203	rc_AI012937_at	NM_013442	1155	N/A
1204	rc_AI008911_at	NM_012470	1156	N/A
1205	rc_AI007875_at	203655_at	1157	N/A
1206	rc_AA997726_at	1136429	1159	N/A
1207	rc_AA963171_at	NM_031210	1161	N/A

* Muscle-specific

Table 13. Human Protein BMNs

SEQ ID NO:	AffyID™	Accession Number	Table 12 SEQ ID NO:	Table 5 sSEQ ID NO:
1208	rc_AI233261_i_at	AAH41809	1115	1179
1209	rc_AI012354_at	E40335	1116	1180
1210	rc_AA955974_at	NP_001842.2	1117	1181
1211	X15939_i_at	P12883*	1120	1182
1212	rc_AA850730_at	Q15040	1121	1183
1213	rc_AI233173_at	P15531	1123	1184
1214	rc_AA998683_at	HHHU27	1125	1185
1215	rc_AA850705_at	NP_116167.1	1127	1186
1216	rc_AA998097_at	P49903	1128	1187
1217	rc_AA997841_at	Q14956	1129	1188
1218	rc_AA800268_at	NP_054901.1	1132	1189
1219	M14050_s_at	AAH20235	1133	1190
1220	L06804_at	P50458	1134	1191
1221	D12769_at	Q13886	1135	1192
1222	AF036335_g_at	P23246	1136	1193
1223	rc_AI030259_at	NP_112496.1	1139	1194
1224	rc_AI639012_at	NP_076947.1	1142	1195
1225	rc_AI237654_at	Q16226	1143	1196
1226	rc_AI228696_at	NP_036238.1	1144	1197
1227	rc_AI177055_at	NP_444278.1	1145	1198
1228	rc_AI059519_at	NP_077300.1	1149	1199
1229	rc_AA899491_at	A41706	1150	1200
1230	rc_AI044635_at	NP_055749.1	1151	1201
1231	rc_AI014091_at	Q99967	1153	1202
1232	rc_AI012937_at	NP_038470.1	1155	1203
1233	rc_AI008911_at	NP_036602.1	1156	1204
1234	rc_AI007875_at	A36353	1157	1205
1235	rc_AA997726_at	Q14690	1159	1206
1236	rc_AA963171_at	NP_112487.1	1161	1207

* Muscle-specific

EXAMPLES

Spinal nerve ligation and artemin treatment

[0057] Male Sragues-Dawley rats were subjected to unilateral spinal nerve ligation (SNL) performed according to the procedure of Kim and Chung (1992) Pain, 50:355-365. Rats with motor deficiency were excluded. The L_5 and L_6 spinal nerves of anesthetized rats were exposed and tightly ligated with 4-0 silk sutures. Sham surgery was identical but without actual ligation.

[0058] Rat artemin (113 amino acids; SEQ ID NO:1237) was isolated and refolded from E. coli inclusion bodies and purified to > 98% homogeneity (Gardell et al. (2003) Nature Med., 9(11):1383-1389). (The amino acid sequence of human artemin is set out in SEQ ID NO:1238). The purified artemin migrated as a reducible dimer by SDS-PAGE and eluted as a single peak (24 kDa) by size exclusion chromatography and by reverse phase HPLC. The purified product was confirmed to contain the characteristic cysteine knot disulfide pattern seen in GDNF, and to be fully active in vitro by assaying receptor binding, cell-based c-RET kinase activation (Sanicola et al. (1997) Proc. Natl. Acad. Sci. USA, 94:6238-6243) and sensory neuronal survival. Artemin (1 mg/kg) was injected subcutaneously on days 3, 5, 7, 10, 12 and 14 following spinal nerve ligation surgery.

Behavioral assays

[0059] Hyperalgesia to thermal stimulation was assessed as described by Hargreaves et al. (1988) Pain, 32:77-88. Latency to withdrawal of a hindpaw in response to noxious radiant heat was determined. A maximal cut-off of 40 sec

prevented tissue damage.

[0060] Tactile withdrawal thresholds were measured by probing the hindpaw with 8 calibrated von Frey filaments (Stoelting, Wood Dale, Illinois) (0.41 g to 15 g). Each filament was applied to the plantar surface of the hindpaw using the up-down method as described by Chaplan et al. (1994) J. Neurosci. Methods, 53, 55-63. Withdrawal threshold was determined by sequentially increasing and decreasing the stimulus strength and calculated with a Dixon non-parametric test (Dixon (1980) Ann. Rev. Pharmacol. Toxicol., 20:441-462).

[0061] Following behavioral confirmation of nerve ligation-induced tactile and thermal hyperalgesia, and efficacy of artemin on neuropathic pain behavior, skin samples were collected on day 14 post-spinal nerve ligation (following artemin injection and behavioral testing) from L4 dermatomes for subsequent gene expression profiling. The skin was shaved to remove as much hair as possible, and 12 skin samples in total were collected and snap-frozen, comprising triplicates from each of 4 groups of rats: (1) vehicle treated + SNL injury (ipsilateral to injury), (2) vehicle treated + SNL injury (contralateral to injury), (3) artemin treated + SNL injury (ipsilateral to injury), and (4) artemin treated + SNL injury (contralateral to injury).

Total RNA Purification

[0062] Snap frozen skin samples were homogenized using an Ultra-Turrax T8 (IKA-Werke, Staufen, Germany) in TRIzol™ reagent (Invitrogen Life Technologies, Carlsbad, CA) according to manufacturer's protocol. 100 µg of total RNA was further purified using an RNeasy™ Mini column (Qiagen, Valencia, CA) according to manufacturer's protocol.

Probe labeling, hybridization and scanning

[0063] The mRNA from skin biopsies samples was profiled on Affymetrix Rat Genome U34A, U34B, and U34C GeneChips™ probe arrays. These arrays contain more than 24,000 mRNA transcripts from gene and EST sequences found in Build 34 of the UniGene™ Database with additional full-length sequences from GenBank™ 110. GeneChip™ probe arrays are made by synthesizing oligonucleotide probes directly onto a glass surface. Each 25mer oligonucleotide probe is uniquely complementary to a gene, with approximately 16 pairs of oligonucleotide probes used to measure the transcript level of each of the genes represented in the array.

[0064] Sample labeling, hybridization, and staining were carried out according to the Eukaryotic Target Preparation protocol in the Affymetrix™ Technical Manual (701021 rev 1) for GeneChip™ Expression Analysis (Affymetrix, Santa Clara, CA). In summary, 5 µg of purified total RNA was used in a 20 µL first strand reaction with 200 U SuperScript™ II (Invitrogen Life Technologies, Carlsbad, CA) and 0.5 µg (dT)-T7 primer (SEQ ID NO:1239) in 1× first strand buffer (Invitrogen, Carlsbad, CA) with a 42°C incubation for 1 hour. Second strand synthesis was carried out by the addition of 40 U of *E. coli* DNA Polymerase, 2 U of E. coli RNase H, 10 U of *E. coli* DNA ligase in 1× second strand buffer (Invitrogen) followed by incubation at 16°C for 2 hrs. The second strand synthesis reaction was purified using the GeneChip™ Sample Cleanup Module according to the manufacturer's protocol (Affymetrix). The purified cDNA was amplified using BioArray™ high yield RNA transcription labeling kit (Enzo Life Sciences, Parmingdale, NY) according to manufacturer's protocol to produce 70-120 µg of biotin labeled cRNA (compliment RNA). Rat Genome U34 A, B, and C

GeneChip™ probe arrays were pre-hybridized in a GeneChip™ Hybridization Oven 640 (Affymetrix) according to the manufacturer's protocol. Fifteen µg of labeled cRNA was fragmented in 30 µL 1× fragmentation buffer 100 mM KOAc, 30 mM MgOAc at 95°C for 35 minutes. The fragmented labeled cRNA was resuspended in 300 µL 1× hybridization buffer containing 100 mM MES, 1 M Na+, 20 mM EDTA, 0.01% Tween™ 20, 0.5 mg/mL acetylated BSA, 0.1 mg/mL herring sperm DNA, control oligo B2, and control transcripts bioB 1.5 pM, bioC 5 pM, bioD 25 pM, and cre 100 pM, and hybridized to GeneChip™ probe arrays according to manufacturer's protocol (Affymetrix, Santa Clara, CA). The hybridized GeneChip® probe arrays were washed and stained using streptavidin-phycoerythrinin (Molecular Probes, Eugene, OR) and amplified with biotinylated anti-streptavidin (Vector Laboratories, Burlingame, CA) (Sigma, Saint Louis, MO) GeneChip™ Fluidics Station 400 (Affymetrix) using an antibody amplification protocol. The GeneChip™ probe arrays were scanned using GeneArray™ scanner (Hewlett Packard, Corvallis, OR).

Data analysis

[0065] Two independent analysis approaches (Rosetta Resolver™ and a proprietary permutation-based Bayesian statistical model) were used to identify bio- and surrogate markers.

[0066] The following analysis techniques were performed using Rosetta Resolver™ software (Rosetta Biosoftware, Kirkland, WA).

[0067] The triplicate samples were considered a single group for ANOVA analyses. The comparisons of interest include the following:

1) Vehicle-treated vs. artemin-treated contralateral dermatomes;

2) Vehicle-treated vs. artemin-treated ipsilateral dermatomes;

3) Contralateral vs. ipsilateral vehicle-treated dermatomes;

4) Contralateral vs. ipsilateral artemin-treated dermatomes.

[0068] A gene list was generated based on those genes whose expression level was found to be significantly different between groups ($p \leq 0.01$). These genes were subsequently tested for significance ($p \leq 0.01$) in fold-change values. The final gene list for each of the 4 comparisons included those genes that passed both criteria. Agglomerative hierarchical clustering techniques (heuristic criteria = average link, similarity measure = Euclidean distance, intensity/Z-score used for clustering) ensured that these final gene lists differentiated well the two populations in each comparison from each other.

[0069] Permutation-based Bayesian Analysis was performed as follows. For all genes, a permutation based approach was used to generate distributions of log ratios of the expression intensity values for all possible pairwise within group (between replicates) and between group comparisons of the samples.

[0070] For example, the 3 replicate rats generated 3 within-group pairwise comparisons for each of the 4 treatment scenarios outlined above. In this way, a total of 12 within-group log ratios and 9 between-group log ratios for the 6 possible between-group comparisons were generated (Table 14). This was done for the A, B, and C chips.

Table 14.

Comparison type	Group 1	Group 2	No. of pairwise comparisons
Between group	Vehicle-treated, ipsilateral (3 replicate rats)	Vehicle-treated, contralateral (3 replicate rats)	9
Between group	artemin-treated, ipsilateral (3 replicate rats)	artemin-treated, contralateral (3 replicate rats)	9
Between group	artemin-treated, ipsilateral (3 replicate rats)	Vehicle-treated, ipsilateral (3 replicate rats)	9
Between group	artemin-treated, ipsilateral (3 replicate rats)	Vehicle-treated, contralateral (3 replicate rats)	9
Between group	Vehicle-treated, ipsilateral (3 replicate rats)	artemin-treated, contralateral (3 replicate rats)	9
Between group	artemin-treated, contralateral (3 replicate rats)	Vehicle-treated, contralateral (3 replicate rats)	9
Within group	Vehicle-treated, ipsilateral (3 replicate rats)	Vehicle-treated, ipsilateral (3 replicate rats)	3
Within group	Vehicle-treated, contralateral (3 replicate rats)	Vehicle-treated, contralateral (3 replicate rats)	3
Within group	artemin-treated, ipsilateral (3 replicate rats)	artemin-treated, contralateral (3 replicate rats)	3
Within group	artemin-treated, contralateral (3 replicate rats)	artemin-treated, contralateral (3 replicate rats)	3

[0071] All ratio calculations were performed using the Affymetrix™ MAS5 application that summarizes the ratios of background corrected intensities (perfect match minus the mismatch intensity values) using an Affymetrix™ proprietary error model described in Affymetrix Microarray Suite User's guide Version 5.0 (2001). Default parameters were used to quantify signal intensities (Alpha1=0.04;

Alpha2=0.06; Tau=0.015; Noise (RawQ)=2.800; Scale Factor (SF)=1.000 Norm Factor (NF)=1.000; Gamma1L=0.0025; Gamma1H=0.0025; Gamma2L=0.003; Gamma2H=0.003; Perturbation =1.1). The summarized signal log ratios with their associated P values were exported for statistical analysis.

[0072] The prior distribution of the log ratios were used to update the P values (posterior probability) of the between group comparison log ratios. Genes with between group log ratio distributions that significantly ($p < 0.05$) differed from the within group distribution of log ratios were selected as differentially expressed genes. The summary log ratio for any comparison was estimated as an error-weighted mean of all the permuted log ratios in that group.

[0073] 308 genes that are affected by spinal nerve ligation injury (vehicle-treated ipsilateral vs. contralateral dermatomes) and that therefore correlate with neuropathic pain behavior are listed in Table 2.

[0074] To identify surrogate markers of artemin neurotrophic activity, genes with specific profiles of interest (e.g., genes that were up-regulated after injury and then down-regulated to normal levels with administration of artemin) were found by intersecting the lists of genes comparing contralateral vs. ipsilateral vehicle-treated dermatomes and vehicle-treated vs. artemin-treated ipsilateral dermatomes. 107 surrogate markers of artemin neurotrophic activity thus identified are listed in Table 6.

[0075] To identify biomarkers of artemin's in vivo biological activity, genes in common on the lists comparing vehicle-treated vs. artemin-treated contralateral dermatomes and vehicle-treated vs. artemin-treated ipsilateral dermatomes were identified. Genes were then identified that are regulated in the same direction by

artemin in the contralateral and ipsilateral dermatomes. 49 biomarkers of artemin biological activity were thus identified and are listed in Table 10. Fig. 7 shows an example of a BMN that has not been confirmed by TaqMan™ analysis.

[0076] To confirm the validity of surrogate markers and biomarkers, 25 preferred surrogate markers of neurotrophic activity and 5 preferred biomarkers were used for sequence analysis to validate the existence of transcripts. The sequence analysis included a BLAST™ search of the Affymetrix™ target sequence against the rat genomic sequence. The genomic locus was then examined for the existence of exons, ESTs, and predicted transcripts. The genes are prioritized based on transcript evidence and subjected to TaqMan™ validation as described below (see, also, Holland et al. (1991). Proc. Natl. Acad. Sci. USA, 88:7276-7280).

TaqMan™ Analysis

[0077] Trizol™ (Invitrogen) purified rat skin RNA was further re-purified using an RNeasy™ Mini kit (Qiagen) according to the manufacturer's protocol. The RNA was digested with Amplification Grade Deoxyribonuclease 1 (Invitrogen) to remove any contaminating DNA, and was subsequently used as a template for cDNA synthesis with a High-Capacity cDNA Archive Kit (Applied Biosystems). The resulting cDNA was used as the PCR template for TaqMan™ analysis.

[0078] The "TaqMan MGB Probe and Primer Design" function of Primer Express 1.5 software (Applied Biosystems) was used to generate primer and probe sequences for Affymetrix target sequences (for example, see Table 15 for rc_AA818804_at RG-U34C, rc_aa818120_at RG-U34C, and X14812_at RG-U34A).

Table 15.

AffyID™	Marker SEQ ID NO:	Amplicon SEQ ID NO:	Forward primer SEQ ID NO:	Reverse primer SEQ ID NO:	Probe SEQ ID NO:
rc_AA818120_at RG-U34C	31, 808	1240	1241	1242	1243
rc_AA818804_at RG-U34C	18, 799	1244	1245	1246	1247
X14812_at RG-U34A	13 813	1248	1249	1250	1251

[0079] Oligomers spanning the PCR amplicon, plus an additional 10 bp on the 5' and 3' ends of each gene were also synthesized. Primers and 6FAM-labeled probes were synthesized by Applied Biosystems, and set up in reactions with the cDNA templates according to standard methods. Reactions were carried out in an ABI Prism™ 7700 Sequence Detector using the default conditions, and the data was analyzed using Sequence Detection Software v1.9.1 (Applied Biosystems). Simultaneous PCR reactions were carried out using a 10-fold dilutions series of the amplicon oligomers to generate a standard curve for each primer and probe set. Cycle Threshold (Ct) values for each experimental reaction were compared to the amplicon standard curve and relative quantities of message were determined. The cDNA samples were also analyzed with TaqMan™ Rodent GAPDH Control Reagents (Applied Biosystems) to determine the amount of GAPDH message in each sample. The samples were normalized by dividing the signal for each of the surrogate marker genes by the signal obtained with the GAPDH control. The results are shown in Figs. 1-6.

[0080] The expression patterns of the genes shown in Figs. 1-6 parallel the results of the Affymetrix analysis. All of these genes are expressed at a low level in

the uninjured state (vehicle/contralateral and artemin/contralateral), are up-regulated in the injured state (vehicle/ipsilateral), and are at least partially normalized following artemin treatment (artemin/ipsilateral). The expression profiles are consistent with these genes acting as surrogate markers of artermin activity in the rat spinal nerve ligation model.

[0081] All references to nucleotide sequences should be understood to encompasses their sequences complementary to a given sequence. All publications and patents and sequences cited in this disclosure by their accession numbers are incorporated by reference in their entirety. To the extent the material incorporated by reference contradicts or is inconsistent with the present specification, the specification will supercede any such material.

[0082] The material submitted herewith on the CD-ROM entitled "Surrogate Markers of Neuropathic Pain," containing file surrmarkers012504.ST25.txt, size on disk 4,515,840 bytes, created on February 20, 2004, is hereby incorporated by reference.

[0083] The specific embodiments described herein are offered by way of example only and are not meant to be limiting in any way. It is intended that the specification and examples be considered as illustrative only, with a true scope and spirit of the invention being indicated by the following claims.